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Studies on Development and Spread of Red Rot in a Sugarcane Plant.

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STUDIES ON DEVELOPMENT AND SPREAD OF RED ROT IN A SUGARCANE PLANT

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ABSTRACT

The present studies were made in order to obtain more information on the development and spread of the red rot fungus in a sugarcane plant. Isolations from the young field-grown shoots were made during the spring. Cultures of the red rot fungus were obtained from leaf scars, leaf sheaths, and internal tissues of these shoots. The average recovery of red rot from leaf scars, leaf sheaths and internal tissues of both plant and stubble shoots, from 1957 to 1959, was 23.1, 28.0 and 1.4 per cent respectively. The buds and bud scales of underground portions of shoots were found to be infected up to 22.3 and 26.3 per cent respectively. Leaf scars and internal tissues of the underground stubble pieces of August planted cane, whose shoots were killed during the winter, also gave red rot.

The shoots grown in the greenhouse from field-inoculated seed cane became infected. Cultures made from leaf scars, leaf sheaths, growing points and internal rolled leaves gave 17.9, 5.0, 3.9, and 1.0 per cent red rot respectively, whereas from shoots of non-inoculated seed cane, the leaf scars, leaf sheaths, growing points and internal rolled leaves gave 4.3, 5.8, 1.5 and 0.2 per cent red rot respectively.

The fungus was cultured from uninjured midribs, inoculated in moist chambers. The recovery after one day was 35.7, after two days 73.8, after three days 79.1, and after four and five days 92.8 per cent. Red rot was reisolated from these midribs after sterilization in a solution of 1:1000 mercuric chloride in 50 per cent alcohol up to 2

hours, which demonstrated the effect of surface sterilization upon the infection of the red rot fungus.

Infection threads from germinating spores on the upper surface of leaves were sometimes observed to penetrate the waxy cuticle. Uninjured, inoculated midribs of greenhouse plants developed pin-point size lesions on some blades. Isolations from these, one month later, gave 60.8 and 68.8 per cent red rot in the varieties Co. 290 and C.P. 44-101. A difference in the percentage of red rot recovery was obtained from inoculation of upper and lower surfaces, being 58.6 and 71.1 per cent respectively.

From leaf sheath inoculations in the greenhouse, first symptoms were observed within 24 hours on leaf sheaths, and lesions developed within six or more days on the blades. When leaf sheaths were inoculated with injury, symptoms appeared on sheaths within 24 hours and lesions on blades after 48 hours. Re-isolations from sheath and blade gave the fungus. Isolations from between lesions did not give the fungus in all cases. Microscopic examinations of hand-made sections from the above leaves, showed atypical spore production inside the vessels and parenchyma cells. In older lesions, mycelium produced more typical spores.

When leaves, with and without sheaths, were placed in a spore suspension for 4 hours, dispersed midrib lesions were developed within 36-48 hours, as a result of spore migration through the ligular region of the leaf.

Leaf sheaths from the field, showing inside reddening, when placed in moist chambers, developed red rot conidia. Reddened midrib lesions, in the early season, did not give the red rot fungus. However, from

apparently healthy leaves from the field in late season, the fungus was often recovered. Isolations from between lesions did not always give the red rot fungus.

INTRODUCTION

Red rot, incited by the organism Physalospora tucumanensis Speg., is one of the most important diseases of sugarcane in Louisiana. It is also an important disease in several of the other cane growing countries of the world and especially in India, where it is the major disease causing heavy losses in the standing cane. In Louisiana, however, red rot is a disease of seed cane and causes a reduction in the number of shoots emerging from the mother stalks in the spring. Due to the red rot epiphytotics in Louisiana, several of the commercial varieties have deteriorated in the past. In 1923-1924, the noble varieties were deteriorated so badly that they had to be replaced. In 1930-1931, due to a severe epiphytotic in P.O.J. 213, it was necessary to abandon this variety. Soon after, the P.O.J. varieties were replaced by Co. varieties and again, in 1940-1941, the variety Co. 290 started showing an increasing susceptibility to red rot (40).

Butler (15), and Butler and Hafiz Khan (17) in India, studied the mode of infection of red rot in detail and reported that the young growing shoots became infected from the seed pieces directly. Workers in the Western Hemisphere, however, could not confirm these results that such a connection between diseased seed pieces and the growing shoots existed. These workers reported that borer holes were the main entrance through which natural infection of the stalk occurred (29, 44, 52). Other workers in India later confirmed Butler's results, stating that the disease was carried over through diseased setts which infected the young growing shoots (21, 43).

Edgerton and Carvajal (35) showed that the red rot fungus could penetrate the uninjured cane tissues by infection threads from appressoria. They reported that no satisfactory infection was obtained from these appressoria on the upper surface of the leaf midribs, while rapid infection inside the leaf sheaths took place. Steib (53, 54), in Louisiana, reported that infection can take place at the nodal region. He reported that the fungus occurred in the stalk in a latent form and isolated the red fungus from the root band regions, buds and leaf scars of apparently healthy stalks. He concluded that the initial point of infection was in the buds and leaf scars and established the fact that the red rot fungus stayed dormant in these tissues. Steib (54, 55) demonstrated that infection threads from the appressoria penetrated the epidermal cell wall of young bud scales 33 hours after inoculation. Chilton and Steib (56) suggested that sugarcane stalks became infected by the fungus sometime during the growing season and that this infection remained in a dormant form until environmental conditions reduced the vitality of the stalks, allowing further invasion by the organism. They (58) also showed that healthy canes of susceptible varieties have a larger amount of the red rot fungus present in leaf scars and bud scales than do resistant varieties.

Workers in Louisiana (7, 9) discovered that spore migration was the basis of longitudinal spread of red rot from one internode to another in the tracheary vessels of the cane stalks. Nesom (49) reported that red rot migrated from one part of the leaf to the other through the ligular region. He suggested that the migration probably took place by means of spores, which were carried through the vascular

bundles in the transpiration stream. Edgerton (33) reported that atypical spores were produced from the mycelium in culture and also frequently within the cells of the sugarcane plant, which after germination readily produced red rot symptoms.

Since it is still not known today, how the red rot fungus spreads from the infected seed pieces to the young shoots and subsequently to the leaves of the mature cane later in the season, an additional study seemed necessary to attempt to demonstrate this important part of the disease cycle.

The present investigation was undertaken to study the development of the red rot fungus from the infected seed cane to the young growing shoots and also its further spread in a mature sugarcane plant.

HISTORICAL REVIEW

The red rot disease of sugarcane, caused by the fungus Physalospora tucumanensis Speg., which is commonly known as Colletotrichum falcatum Went, was first described in Java by Went in 1893. He gave an accurate account of the red rot symptoms which are characterized by red discoloration of the internodal tissues and the presence of white typical blotches (Plate II). In the same year Cobb (25) recorded the disease in New South Wales, Australia and stated that the damage done by it was considerable. The condition he described was red rot although his drawings of the sporulating fungus were not strictly accurate. Towards the end of 1893, Massee (46) at Kew Gardens isolated Colletotrichum falcatum from cane received from Barbados, West Indies. He considered it to be the cause of root rot and attributed the stalk rotting to a new fungus Trichosphaeria sacchari Massee. This led to considerable confusion in regard to the identity of red rot in the West Indies. Howard (41) reported that this destructive disease in the West Indies which was commonly called "rind disease," was in reality red rot, and found that invasion by the rind disease fungus Melanconium sacchari Mass. was secondary following severe injury by Colletotrichum falcatum.

It is evident from Benson's (12) report that the disease had been present in the Madras Presidency for some years and was probably well established in other parts of India also. Barber's early records (10, 11) indicated that it was a very important sugarcane disease in Madras. He found it severe in regions where borers were almost entirely

absent and he was the first to note that the thin varieties of cane were more resistant than the noble varieties. Butler (15) studied the disease in detail in India, particularly the causal organism and mode of infection. He was the first to give the name "red rot" to the disease.

Lewton-Brain (44) reported the red rot disease in Hawaii in 1908. Thefirst specimen of red rot found in the United States was collected at Audubon Park, New Orleans in February, 1908. During the fall and winter 1909-10 it was first found on a plantation in Orleans Parish, Louisiana, and from Georgia. The disease was first reported by Edgerton in 1910 (27). The disease has now been reported from all the major sugarcane producing countries of the world.

Red rot has been recognized as a disease causing severe losses to the sugarcane crop. Butler (15) in 1906 reported severe losses from red rot disease of sugarcane in Bengal, India. As early as 1910, Edgerton (28) reported the deterioration of seed cane due to red rot both in Louisiana and Georgia. Edgerton (29) mentioned various kinds of losses due to red rot, such as loss in stand, killing of young plants, injury to the leaves, and a loss in per cent of sucrose.

In 1920 Edgerton and Moreland (36) reported that the red rot was one of the important causes of poor stands of canes in Louisiana. According to Edgerton, Taggart and Tims (38) the season of 1923 was one of the worst red rot epiphytoties in the history of Louisiana. This very heavy red rot infection was also responsible for the very poor stands in 1924, the year of the first severe cane failure. The young cane plants died out to such an extent that stands of 40 to 50 per cent were the rule.

According to Tims and Edgerton (59) the stands of cane again in 1929 were reduced nearly 50 per cent by the disease and a considerable portion of the Louisiana Purple produced during 1927 was so severely rotted that it was not worth sending to the mill. By 1929 P.O.J. 213 occupied one-third of the entire sugarcane acreage in Louisiana (32). In 1930, there was a severe epiphytotic in P.O.J. 213, the year of its first disastrous failure (32) and the result was a sharp decline in acreage of P.O.J. 213. Bourne (13) reported an epiphytotic of red rot in P.O.J. 2714 in Southern Florida, which caused 30 per cent loss in tonnage and reduction of 50 per cent in the sucrose of harvested cane.

Edgerton, Forbes and Mills (37) stated that the most serious effect of red rot is the killing of the buds on the seed cane, which means poor germination, poor stand in the field and decreased tonnage at harvest time. The reported reductions in stands and yields ranged from none in resistant varieties to severe in susceptible varieties. Chilton and Mills (20) showed the effect of red rot on the yields of different cane varieties in inoculated and uninoculated cane in the field. Nine cane varieties yielded an average of 20.7 tons per acre in the check (not inoculated), while 11.4 tons per acre was the average in inoculated cane.

Red rot has also been reported to be an important factor in stubble deterioration (30, 39). They found that many of the eyes were killed before they had a chance to germinate and some of the young shoots that did emerge from the ground died during the spring months. Hughes (42) reported that many diseased fields of Co. 290 in the Moreton Area, Australia had to be ploughed out for this reason. During 1940-41 reports regarding the deterioration of the planted seed cane and stubble seed

pieces of Co. 290 appeared from Louisiana. The deterioration was considered to be due to red rot (36) and in subsequent years Co. 290 started showing an increasing susceptibility to red rot and ultimately deteriorated.

Padwick (50) reported that red rot was in many places the greatest obstacle to succesful cultivation of sugarcane in India, an area where big-barreled canes are grown. According to him the disease was present in the Godavari delta in Madras in 1902. In 1906, it was reported in severe form in Champaren area of Bengal. In 1922, there was a severe epiphytotic in Jammu. In 1932, a severe outbreak occurred in Pusa on Co. 210. In 1935, a severe epidemic occurred in eastern Uttar Pradesh. Padwick found that the majority of diseased canes showed no sign of borer damage. He reported an epiptytotic in the medium thick cane, Co. 213, Chona (21) reported that red rot was by far the most important disease of sugarcane in India. According to him, Butler reported that red rot often is the limiting factor in the successful cultivation of heavy yielding canes, such as would enable India to hold its own against other sugar producing countries, like Java and Mauritius, The red rot epiphytotic in 1938 to 1941, resulted in the widespread failure of Co. 213, the chief commercial variety in the Northern Indian white sugar belt. The estimated loss of 75,000 tons in sugar production in India during 1938-39 was ascribed to the ravages of red rot.

Went (63) first reported that the red rot causes reductions in recoverable sugar at the factory because of the inversion of sucrose in the stalk, and found that the disease greatly lowered the quality of mill cane. Butler in India (15) pointed out that the damage was due to

the inversion of sucrose and not the actual consumption of sugar by the fungus. Similar results were reported by Edgerton (29) from Louisiana and Lewton-Brain (44) from Hawaii. In the 1927 epiphytotic in Louisiana, the reductions in the sucrose in the juice of the noble varieties were as high as 33 per cent (6). According to McKing and Fort (47) not only the quantity but also the quality of the juice is lowered. They reported that the disease decreased juice extraction, affected the percentage of solids and sucrose in the juices, and lowered the purity and resulted in other deleterious chemical changes. The chemical changes in the very susceptible P.O.J. 213 variety were greater than in the resistant Co. 281.

There are different points of view in the literature regarding the manner in which the red rot fungus enters into the sugarcane stalks. One of the prominent ones held that some kind of wound is required before infection would occur. Went (63) was the first to conclude that natural infection occurred chiefly through the holes made by boring insects. Butler (15) and Butler and Hafizkhan (17) however, found little or no natural infection through borer holes in India. That borer holes were the main source of entrance through which natural infection of the stalk would occur was also shown by Lewton-Brain from Hawaii (44), Edgerton from Louisiana (29) and South and Dunlop from West Indies (52). Chona (21) found no association of borers with red rot in India and stated that borers play little part in starting the red rot infection in cane or its spread in the crop.

The direct infection of the young growing shoots from the diseased seed piece has been reported with divergent opinions in the literature.

Raciborski (51) from Java reported that most of the infection of growing plants can be ascribed to direct mycelium connections between the stalks and diseased cuttings. Butler (15) in 1906, claimed that the mycelium of the red rot fungus passed directly from the diseased seed pieces into the young plants and most of the growing plants became infected in this manner. Edgerton (29) from Louisiana and South and Dunlop (52) from West Indies were unable to confirm that a direct mycelium connection existed between the seed cuttings and growing plants. After such conflicting reports Bulter and Hafizkahn (17) again worked on the whole problem in India. They conducted very extensive studies on the mode of infection of red rot and confirmed the spread of the fungus from the seed piece to the growing stalk. They planted alternate rows of diseased and healthy seed cuttings, and after three months, the number of sound shoots counted in eight trenches each from healthy and diseased seed, were 679 and 117 respectively. They reported that the field in which the experiment was conducted had not been under cane in recent years and no other cane had been grown the previous year within about half a mile. They showed definitely that the plants do become infected from the diseased seed. The course of infection up into the stem can be traced in many cases and direct connections between the mycelium in the seed cane and that in the new shoots established. Later Kulkarni (43) also confirmed Butler's results in India. Chona (21) also reported that studies on the mode of infection confirmed Bulter's view that the disease is carried over through diseased setts. The setts inoculated with the fungus or from diseased canes, gave poor germination and heavy red rot infection in the resulting crop. He stated that the infection lesions were often observed passing from diseased mother setts

to new shoots in varieties Co. 213, Co. 223, Co. 299 and Co. 331. Edgerton and Moreland (36) stated that conditions in India were radically different from those in the West Indies and Southern United States. Just why the fungus acted differently in the different countries was not known. It may be, they said, that there were different strains of the fungus in the various countries or that there were differences in varietal susceptibility or climatic conditions.

Steib (54) reported that healthy shoots attached to the seed piece developed the disease in storage and infection occurred at the base of the shoot at the point of contact with the old bud scale. McMartin (48) observed in Natal that the young shoots are surrounded at their base by disintegrating infected material which might prove a source of infection for penetrating the young stem from the outside. He found that the leaf sheaths had discolored areas in them which proved to be lesions of red rot. These discolored areas were found penetrating to the stem. He suggested that here the fungus perhaps can grow from the infected material in the ground, up the outside stem or the leaf sheaths and ultimately penetrate the stem itself.

The root primordia (6, 17), leaf scars (28, 41, 63), mechanical wounds, and growth cracks (17) have also been described as point of entrance for the red rot fungus. Butler and Hafiz Khan (17) reported that infection can also take place through the cut ends of the setts. According to these workers, old leaf scars were not readily penetrated, and since the leaf scars are normally not exposed until the leaf has completely withered, they were not considered an important point of entrance. Abbott (6) also reported that the fungus could enter the stalk through the cut ends. Edgerton and Moreland (36) pointed out that the yeasts

which usually invade the cut ends serve to prevent the growth of the fungus.

Steib (53) reported that infection could take place through the leaf scar and that the leaf scar became infected during the growing season before the leaf had completely withered. He isolated the red rot fungus from apparently healthy canes from the root band region and bud after 24 hours sterilization in a solution of bichloride of mercury followed by calcium hypochlorite. He showed that infection can occur at the nodal region independent of borer holes. Edgerton and Moreland (36) earlier postulated that the fungus may gain entrance to the seed cane through the very small thin places in the rind of the nodes where the young roots emerged. Though this was first suggested as a possible mode of entry by Butler and Hafiz Khan (17), latter Abbott (6) also demonatrated that the fungus entered through the root band region. Steib (54, 55), though he was able to isolate the organism from the root band region even after 24 hours immersion in a solution of bichloride of mercury, eventually reached the conclusion that the initial point of infection was probably the buds and leaf scars, and not the root band region. Padwick (50) studying the possible modes of entry of the fungus into the stalk in India reported that the stalk may become infected through borer holes, from the mother stalk into the new shoot, through the root primordia and leaf scars, through the cut ends of the seed pieces and through miscellaneous injuries.

The spread of the fungus in the stalks has been attributed to spores carried through the vascular bundles by Atkinson (7) and Atkinson and Edgerton (9). They showed that spores of the red rot fungus migrated

through the large ducts in the fibro-vascular bundles. These workers demonstrated that in varieties such as Co. 281 with open ducts the spores were able to migrate through several internodes, while in varieties such as C.P. 29-116 with cross walls in the ducts, spores usually remained confined to a single internode. Varma and Mital (60) reported that rapid spread of red rot in sugarcane stalks is partly due to the migration of spores through the open vascular bundles at the nodes. Vascular strands of the nodal region of Co. 393 showed the presence of septa stopping the flow of India-ink through them, which indicated that spores carried through xylem vessels would be stopped by septa. Nesom (49) reported that the organism migrated from one part of the leaf to the other through the ligular region, but no such migration was obtained between the leaf and the stalk. He suggested that the migration probably took place by means of spores which were carried through the vascular bundles in the transpiration stream.

In recent years it has been shown that the red rot fungus could penetrate the uninjured tissues by infection threads from appressoria (35). It was reported from Louisiana that under ordinary field conditions, every part of the surface of the plant bears appressoria capable of originating an infection. Edgerton and Carvajal (35) found that no satisfactory infection was obtained from these on the upper surface of midribs, while inside of leaf sheaths infection was obtained easily and rapidly and that within 3 to 4 days lesions extended entirely through the leaf sheath. Steib (54, 55) demonstrated that appressoria formed and infection threads penetrated the epidermal cell wall of young bud scales 33 hours after inoculation. He also reported that removal of

leaf sheaths from the stalks before they became infected greatly reduced the amount of latent infection in the nodal region. Steib and Chilton (56) indicated that the leaf scar tissue and bud contain the red rot fungus even after surface sterilization and the removal of the dead outer tissues. They suggested that the sugarcane stalks standing in the field might be infected with the red rot fungus previous to planting, perhaps in a dormant form, and that the red rot develops from these centers of infection into the interior of the cane stalk when environmental conditions are favorable. They (57, 58) also showed that apparently healthy stalks of susceptible varieties of sugarcane have a larger amount of red rot fungus present in the leaf scars and bud scales than do resistant ones. The presence of the red rot fungus in a dormant or latent form in the sugarcane stalk was first suggested by Steib (53).

Several workers have also reported the possibility of red rot infection through soil. Butler (15) stated that the fungus can live in the soil or on decaying leaves in the absence of cane but indicated that it could not so survive for more than 3 to 4 months. Butler and Hafiz Khan (17) reported that the fungus died out rapidly in moist soil, but cultures kept dry and exposed to the air retained their viability for 5 months. However, Abbott (1, 6) failed to isolate the fungus directly from the soil and concluded that the soil as a source of inoculum is relatively unimportant in the life history of the disease. He stated that seed cane in Louisiana was commonly planted with leaves and sheaths adhering to the stalk. This meant an abundant supply of red rot spores and mycelium went into the soil with seed pieces, in addition to that which already may have established itself within the stalks. Dastur

(26) reported that experiments have demonstrated the transmissibility of infection on a considerable scale through contaminated soil and irrigation water in nodal regions of the cane plant. The fungus was found to survive for about 6 months in a fallow land. Chona and Padwick (24) reported the infection of young shoots from red rot cane debris applied in the soil at the time of planting of seed cane in pots as well as in the field. Chona (21) demonstrated that a considerable red rot infection can take place through soil with red rot affected debris or the spores and mycelium of the fungus, even when healthy setts are planted. He also reported irrigation water as a source of red rot infection.

The perfect stage of the red rot fungus was first reported from Louisiana in 1943 and described in 1944 by Carvajal (18), and Carvajal and Edgerton (19). Later, it was also reported from other sugarcane growing countries. Ling and Ma (45) and Wang (62) studied the perfect stage in Formosa (Taiwan). Carvajal and Edgerton (19) reported the occurrence of perithecia most commonly on dead and dying leaf blades and leaf sheaths of the affected canes. Leaves on shoots killed by too much crowding frequently have been found covered with perithecia. These perithecia had been previously found by Spegazzani in Argentina in 1896, according to Carvajal and Edgerton and described as Physalospora tucumanenis, but were not associated with the red rot fungus. Recently, von Arx and Müller (61) described the perfect stage of the red rot fungus as Glomerella tucumanensis. On the basis of conidial form, however, they were not able to separate this species from Glomerella cingulata. The name, Glomerella tucumanensis has now been adopted by the British Commonwealth Mycological Institute.

The sudden decline of cane varieties, especially P.O.J. 213 in Louisiana resulted in speculation of the possibility of the existence of specialized races of the fungus with varying powers of pathogenicity. Tims and Edgerton (59) were the first to show that all cultures of the red rot fungus did not show the same pathogenicity to different sugarcane varieties. However, the evidence did not indicate that these were distinct physiologic strains. Abbott (2, 4) made preliminary investigations of physiologic races. Later, (6) he gave evidence of the existance of specialized races and indicated that there are at least two distinct strains of the red rot fungus, light and dark strains, attacking cane varieties differently. He believed that the severe epiphytotic in P.O.J. 213 was due to the build up of a particular strain. He determined the comparative virulence of both these strains on a resistant host Co. 281 and a very susceptible variety, P.O.J. 213, and found that the isolates of the light race from Louisiana were more virulent in general than those of the dark, and the latter were more virulent than either the light or dark race isolates from the sirup producing states. Although well defined physiologic forms could not be differentiated, a degree of parasitic specialization within each cultural race was demonstrated (6).

Abbott (6) reported that some light type cultures showed a tendency to grade to the color of the dark types and occassionally the dark colored ones tended toward the lighter color of the light types. He suggested a separate classification for these intermediate types. Chona and Hingorani (22) observed the behavior of one dark colored isolate and two light colored isolates. They found that the light colored isolate gave rise to mutants of dark types, but the dark colored isolate and the

dark mutants remained stable. They further reported that the frequency of mutation increased with the age of the culture.

Edgerton and Carvajal (35) studied the host-parasite relationship of the red rot fungus in detail, including cell wall penetration and the reaction of the host cells to the invading mycelium. The more acceptable theories regarding the cell wall penetration are that either the fungus produces an enzyme which dissolves the cellulose in advance of the invasion thread or that the fungus actually exerts enough pressure to force the hypha through the cellulose wall. In recent years the latter theory has perhaps become more generally accepted. However, these workers could not determine how the infection thread penetrates the wall.

The parenchyma cells in which the sucrose is stored are large and their cell walls are firm, relatively thin and with numerous pits. According to these workers the mycelium from spores germinating in the ducts of the fibro-vascular bundles or from other centers of infection grows out very rapidly through a number of layers of cells. The mycelial threads after entering a cell do not branch profusely there but continue to grow directly across the cell to the opposite wall and then into the adjoining cell (35).

In advance of the mycelium the protoplasm of the host cell changes in color and a gummy dark red material oozes out of the cells and fills the intercellular spaces. This zone in advance of the mycelium turns red, due to the presence of a dye which is absorbed by the cell walls. The growth of the advancing mycelium is considerably checked by the red zone. It is however, they reported, not known if this check in growth is due to the presence of some counteracting toxic substance, to

the plugging of the cell walls by the gummy material, or to some other reason (35).

The red zone develops into a typical or characteristic lesion of the red rot with a white or straw-colored spot with mycelium surrounded by a distinct red border. Mycelium advances slowly into the red zone. They reported that the red zone forms quickly in resistant varieties and more slowly in susceptible ones. Apparently in all cases studied by them, the mycelium passes from cell to cell through pits of the cell walls in the internal parenchyma. The mycelium varies from the relatively small and scattered hyaline threads in the newly infected regions to a network of thick brown threads in the old lesions (35).

In the inoculated leaf sheaths, the infection threads were sent out from the contact surface of the appressoria and penetrated the cell walls of the epidermis. It is not known they reported how these threads penetrated the cell walls of the epidermis since pits were not observed in the outside walls of the epidermal cells. After entering a cell of a second layer, the infection thread widens out to the normal small type of hyphae. Then hyphae, however, continued to grow directly towards the center of the leaf sheath, often elongating parallel to the cross walls of the cells. Often, a somewhat flattened body was visible when the infection thread first entered the interior of some of these cells. After growing for a period within the host tissues the red rot fungus breaks out to the surface and produces its spores in an acervulus (35).

Butler (15) was the first to demonstrate that isolates of the fungus obtained from leaf lesions are capable of producing red rot in

the stalks and the stalk isolates of producing the disease in leaves. According to Abbott (6) no idrect mycelial connection between leaf and stalk lesions has ever been reported in the literature, while it may occur but certainly is very rare. Abbott (6) reported that a lesion grows from a single point of infection and is normally continuous along the midrib, but sometimes it is broken up into a succession of red blotches alternating with apparently healthy tissue. He said that microscopic examination of these areas will sometime reveal strands of the fungus mycelium connecting the reddened spots but frequently, however, no such connecting is apparent. As a result of the demonstration of the movement of conidia in the stalks of sugarcane through the bundles in the transpiration stream and lodging at intervals and initiating new points of infection, Atkinson and Edgerton (9) suggested that a similar movement in the leaves may be responsible for the discontinuous lesions.

Edgerton (33) reported that besides typical conidia, conidia of another type are not uncommon. These are considerably smaller than the typical ones, being long and narrow in shape and either straight or slightly fusoid. They form on the mycelium in culture and frequently within cells in the interior of the cane plant, especially in cells near the fibrovascular bundles. Sometimes certain cells are packed with these spores. These are sometimes spoken of as "off-type" or "atypical spores," he said and they germinate readily and produce typical red rot colonies.

MATERIALS AND METHODS

The different varieties of sugarcane used in these studies were rated from very resistant to susceptible. The variety Co. 290, susceptible to red rot and C.P. 44-101, resistant, were mostly used in the greenhouse inoculation experiments. Besides these, other varieties used in the study of red rot in the field were:

P.O.J. 213	(S)	C.P. 34-120	(S)
Co. 281	(R)	C.P. 36-13	(VR)
N.Co. 310	(S)	C.P. 36-105	(R)
		C.P. 43-47	(MR)
C.P. 807	(MS)	C.P. 47-193	(MR)
C.P. 29-116	(R)	C.P. 48-103	(MR)
C.P. 29-120	(MS)	C.P. 52-68	(R)
C.P. 29-320	(MS)	C.P. 53-1	(MR)

Preparation of the Culture Media

Oatmeal agar medium was used for culturing the fungus from diseased or healthy parts of the sugarcane plant. This was prepared by using 65 grams of oatmeal and 17 grams of Bacto agar per liter of water. The oatmeal was placed in a liter flask and 500 cc of hot water from the tap was added and left for about 15 to 20 minutes. This was then filtered through cheese cloth. The Bacto agar was boiled in 500 cc of water in another flask. The filtrate of oatmeal extraction and water agar were then thoroughly mixed and sterilized by autoclaving for 30 minutes at 15 pounds pressure. The agar was acidified by adding one drop of 50 per cent lactic acid to a plate, to reduce the bacterial contamination.

Isolation From the Young Shoots

The presence of red rot fungus in the young growing shoots was

determined by a plating technique. The shoots of both plant and stubble canes were dug in the spring from the fields of different plantations in Louisiana, and were brought into the laboratory. The shoots were removed from the mother stalks and washed thoroughly with water. Before plating, they were immersed from 5 to 10 minutes in a 1:1000 solution of mercuric chloride in 50 per cent alcohol. This sterilizing solution was prepared by adding two tablets of mercuric chloride to 1000 cc of 50 per cent alcohol. After surface sterilization, the treated shoots were transferred to a saturated solution of calcium hypochlorite, made by adding 40 grams to a liter of water, and remained in it during the time isolations were being made.

The leaf scars, leaf sheaths, internal tissues, buds and bud scales, stubble pieces and roots were the tissues of these young shoots plated subsequently to sterilization. A pair of sterile forceps and a knife were used in removing these tissues from the shoots. Four to five and sometimes six pieces were plated in a Petri dish. They were numbered according to shoot and tissue pieces. These plates were then kept in a room with a temperature of 70° F for 6 to 7 days and then red rot isolations were recorded.

Growing of Shoots in the Greenhouse From Field Inoculated Seed Cane

Single eye pieces of varieties Co. 290 and C.P. 44-101 were planted in greenhouse flats, containing a soil mixed with peat moss. Inoculated and non-inoculated seed pieces of each variety were used in these experiments. The seed pieces were obtained from sugarcane plants inoculated

in the field by spraying with spore suspensions of the red rot organism. Spraying began June 4 and continued at weekly intervals thereafter, until the stalks were cut for planting on July 24. The non-inoculated seed pieces were cut from stubble cane. Four to five lower nodes of each stalk with buds were used. These single eye seed pieces were sterilized in a 1:1000 mercuric chloride solution for 5 minutes and then washed with water before planting. Twelve of these one-eyed pieces were planted in each flat. When germinated, these were dug from the flats, washed thoroughly with water to remove the soil, sterilized for 5 minutes in accordance to the standard procedures as described before, and plated on oatmeal agar.

Inoculation of Leaf Midribs in Moist Chambers

Healthy sugarcane leaves were collected from field and greenhouse grown plants. Seven inches long midrib pieces were cut from each leaf. They were first washed thoroughly with water, then sterilized for 5 minutes in a solution of 1:1000 mercuric chloride and washed again with water. The cut ends of the midrib pieces were dipped in a melted wax, before placing in moist chambers. Spore suspensions were prepared from seven days old cultures of the red rot fungus. The upper surface of the leaf was inoculated by placing a spore suspension on the midrib with an eye dropper. Inoculations were made on both injured and non-injured midribs. The injury was made by puncturing the midribs with a needle. Checks were kept for each treatment.

After one, two, three, four and five days, the midribs were plated on oatmeal agar. Before plating, they were washed under running tap water to remove any inoculum left and then sterilized for 12 minutes in

a solution of 1:100 mercuric chloride in 50 per cent alcohol, followed by a solution of calcium hypochlorite. A pair of sterile forceps and a knife were used and five pieces were cut at random from each midrib and plated in a Petri plate.

In another experiment, non-injured inoculated midribs were used for isolations after sterilizing for 5, 10, 15, 20, 30, 40, 50, 60 and 120 minutes in the above mentioned solutions. The plates were incubated at room temperatures and the records were made after 7 days by counting thered rot colonies.

Inoculations of Leaf Midribs of Greenhouse Plants

Leaf midribs of Co. 290 and C.P. 44-101 were inoculated with spore suspensions of red rot. Injured and uninjured midribs were inoculated on both upper and lower surfaces. Checks for each treatment were kept. Injury was made by puncturing with a needle. Moist pieces of absorbent cotton were dipped in spore suspensions and then placed on the surface as a method of inoculation. Pieces of dry cotton were wrapped around both ends of the inoculated midribs to hold and not let drip the spore suspensions on other parts of the plant. The inoculated part was covered by folding a piece of wax paper around and paper clips were placed at both ends. This made an artificial moist chamber on the leaf (Plate 1). After 48 hours, the wax paper and cotton were removed. The disease development was observed regularly. Isolations were made from the inoculated leaf midribs.

Inoculation of Greenhouse Plants by Spraying

Plants of Co. 290 and C.P. 44-101 were inoculated in the greenhouse by spraying with spore suspensions. Injured and uninjured plants were inoculated. Check plants were kept for each treatment and sprayed with sterile water. The plants were placed in a greenhouse moist chamber for 48 hours and then were removed to the greenhouse benches. Observations were made for disease development.

Inoculation of Leaf Sheaths

The plants used for leaf sheath inoculations were grown in pots in the greenhouse. The varieties used for these inoculations were mostly Co. 290 and C.P. 44-101 but C.P. 36-105 was also used. When leaf sheaths started pulling away from the stalks, they were inoculated by placing spore suspensions of the red rot fungus between the leaf sheaths and the stalk. Two to four leaves were inoculated on one plant. The spore suspensions were prepared in water from a 6 to 7 days old cultures. Each leaf inoculated was labeled. Regular observations of the symptom appearance on leaf sheaths and the spread of the fungus to the midribs were made up to one month. The fungus was reisolated from such leaves. Check, non-inoculated plants were kept of each variety. Injured leaf sheaths were also inoculated by pricking with a fine needle and adding spore suspensions.

Hand sections were made of the midribs which showed the disease symptoms. Leaf sheath sections were also made. Sections were also made from portions of the midrib showing no symptoms. These sections were stained with cotton blue and were studied under the microscope.

Spread of Red Rot in Detached Leaves

Healthy leaves of Co. 290 and C.P. 44-101 were brought to the laboratory. The leaves were cut with and without the leaf sheaths under water with a pair of scissors. These leaves were transferred to the flasks containing spore suspensions and left for four hours. They were then removed to other flasks containing water and kept for 7 to 10 days. Checks for each treatment were kept only in water. Symptoms were observed and the spread of the red rot fungus in midribs was finally recorded after 7 days.

Presence of Red Rot Infection Inside the Leaf Sheaths

Leaves showing reddening inside of the leaf sheaths were collected from six different varieties of cane in the field. These were brought to the laboratory and washed with water. They were sterilized in 1:1000 mercuric chloride for 5 minutes and then were placed in moist chambers. After five days, scrapings of the leaf sheaths were made and studied under the microscope for the presence of red rot spores.

Isolations from Healthy and Diseased Midribs

Diseased and healthy midribs were collected from the field during the growing seasons of 1957, 1958, and 1959, from which isolations were made. The leaf midribs were first washed and then sterilized in a solution of 1:1000 mercuric chloride in 50 per cent alcohol for 5 to 7 minutes and transferred to a solution of calcium hypochlorite. Five to six pieces were cut at random from each leaf midrib and plated on oatmeal agar. Plates were kept at 70° F for incubation. After 7 days, the colonies of red rot and other fungi were counted and recorded.

EXPERIMENTAL RESULTS

Red Rot Infection and Development in Young Shoots Under Field Conditions

These studies were undertaken in order to determine the presence of red rot infection and its development in young shoots grown from infected seed cane under field conditions. In the literature, it has been reported by several workers that the young emerging shoots become infected directly from the seed cuttings. A latent type of bud infection with red rot is common in sugarcane seed pieces planted in Louisiana. It was thought that this might be an important factor in the development of the causal organism within the young plants growing from infected seed cane. Isolation studies were made from different parts of the young shoots to determine the presence of the red rot fungus.

Occurrence of Red Rot in the Shoots of Plant Cane.

Isolations were made from young shoots of plant cane of the variety C.P. 44-101 by plating leaf scars, leaf sheaths, and internal tissues. These shoots were collected from different locations in Louisiana during the months of March and April, 1957. They were sterilized by immersing in a solution of 1:1000 mercuric chloride in 50 per cent alcohol at periods varying from 5 to 10 minutes, followed by immersion in a saturated solution of calcium hypochlorite. Platings were made on oatmeal agar. The results are summarized in Table I.

The red rot fungus was isolated from leaf scars, leaf sheaths and internal tissues of the young shoots (Plate III). A difference in the number of isolates from different plantations was found. The results

Table I. Occurrence of red rot in leaf scars, leaf sheaths, and internal tissues of the young shoots of plant cane of the variety C.P. 44-101 collected from different locations during March and April of 1957.

Location	Date of Plating	No. Shoots Plated	Leaf Scar		Leaf Sheath		Internal Tissue	
			Number Plated	Per cent Red rot	Number Plated	Per cent Red rot	Number Plated	Per cent Red rot
L.S.U. Expt. Sta.	March 17	33	51	11.8	106	10.4	495	1.2
Allendale Plantation	March 30	35	105	4.8	105	5.7	175	0.0
Westover Plantation	March 31	30	114	17.5	114	22.8	150	0.7
L.S.U. Expt. Sta.	April 6	20	60	25.0	60	13.3	100	0.0
Halfway Plantation	April 10	25	134	61.2	133	54.1	149	0.0
Barozza Plantation	April 12	12	36	19.4	38	28.9	96	3.1
Elm Hall Plantation	April 20	14	57	82.5	45	68.9	84	0.0
L.S.U. Expt. Sta.	April 26	15	48	16.7	48	16.7	90	0.0
Delphine Plantation	April 27	21	30	50.0	126	44.4	24	0.0
L.S.U. Expt. Sta.	April 27	10	24	0.0	24	0.0	40	0.0*
L.S.U. Expt. Sta.	April 30	12	0	0.0	0	0.0	30	26.7**

*Seed pieces with shoots attached stored for two weeks at 70° C.
**Seed pieces with shoots attached stored for three weeks at 70° C.

show that the shoots which gave a higher percentage of red rot from leaf scars also gave a higher percentage from leaf sheaths. The per cent of red rot fungus in leaf scars and leaf sheaths was 82.5 and 68.9 respectively, in shoots obtained from Elm Hall Plantation. From Halfway Plantation the shoots gave 61.2 per cent red rot from leaf scars and 54.1 per cent from leaf sheaths. The shoots from Allendale Plantation gave the lowest percentage of red rot, being 4.8 per cent from leaf scars and 5.7 per cent from leaf sheaths. The highest infection of the fungus in internal tissues was 3.1 per cent from Barozza Plantation. When shoots were left attached to seed pieces and stored for two and three weeks, no fungus was isolated from leaf sheaths and leaf scars, since leaf sheaths were completely dry. A reddening of the tissues connecting seed pieces and shoots was observed. From internal tissues of such shoots 26.7 per cent red rot was isolated.

During the month of March, 1957, 98 shoots from three different plantations were plated and the average percentage of red rot was 11.4 per cent from leaf scars and 12.9 per cent from leaf sheaths. From six different plantations during April of the same year, 107 shoots were plated and average percentages of red rot were 42.5 from leaf scars and 37.7 from leaf sheaths.

Results from Plant Cane Isolations, 1958, are Given in Table II.

The shoots of ten different varieties were collected from 18 different locations during the spring of 1958. The red rot fungus was cultured from internal tissues of the varieties C.P. 44-101, Co. 290, and C.P. 36-105, results being 2.9, 4.2 and 5.0 per cent respectively from the pieces plated. The leaf scars of C.P. 48-103 gave 60 per cent red

Table II. Occurrence of red rot in leaf scars, leaf sheaths, and internal tissues of young shoots of plant cane collected from 18 different plantations during February, March and April of 1958.

Variety	No. Shoots Plated	Leaf Scar		Leaf Sheath		Internal Tissue	
		Number Plated	Per cent Red rot	Number Plated	Per cent Red rot	Number Plated	Per cent Red rot
C.P. 44-101	129	228	17.4	390	27.2	466	2.9
N.Co. 310	25	39	0.0	75	0.8	65	0.0
C.P. 36-105	20	30	6.7	65	9.2	100	0.0
Co. 290	11	21	28.6	33	39.4	48	4.2
C.P. 36-13	8	24	4.2	24	16.7	40	5.0
C.P. 807	8	-	-	24	20.8	40	0.0
C.P. 53-1	7	21	19.0	21	19.0	35	0.0
C.P. 48-103	5	15	60.0	15	20.0	25	0.0
C.P. 47-193	3	13	0.0	9	0.0	5	0.0
C.P. 43-47	2	6	0.0	6	33.3	-	-

rot, Co. 290 gave 28.6 per cent, C.P. 53-1, 19.0 per cent, and C.P. 44-101, 17.4 per cent. The highest percentage of red rot, 39.4 was from leaf sheaths of Co. 290; followed by 33.3 from C.P. 43-47 and 27.2 from C.P. 44-101. The lowest percentage from leaf sheaths was 0.8 from N.Co. 310 and none from C.P. 47-193. The total number of shoots from which isolations were made was 218 and the average percentage of red rot of all varieties from leaf scars and leaf sheaths was 15.3 and 18.6 respectively.

Results of 1959 Plant Cane are Presented in Table III.

Table III records the isolation from leaf scars, leaf sheaths and internal tissues of shoots of plant cane of seven different varieties during the spring of 1959. These were collected from different locations. The only internal tissues that gave red rot were from shoots of the varieties C.P. 44-101, N.Co. 310, and C.P. 36-13 and the percentages were 2.3, 2.5 and 4.1, respectively. C.P. 36-13 gave 24.4 per cent red rot from leaf scars, and C.P. 44-101 gave 20.6 per cent. Leaf sheath pieces of N.Co. 310 gave 39.2 per cent red rot, C.P. 36-13 gave 31.0 per cent, C.P. 36-105 gave 28.0 per cent and C.P. 44-101 gave 25.3 per cent.

Occurrence of Red Rot in Shoots of Stubble Cane.

The young shoots from five different varieties and four different locations were collected during March and April of 1957. No varietal difference was observed as to the infection of red rot in the young shoots. A higher percentage of red rot in leaf scars was generally followed by a higher percentage in leaf sheaths. The shoots of the variety Co. 290 gave 29.2 per cent red rot from leaf scars. The highest percentage of red rot recovered from leaf sheaths was 43.4 from shoots of the

Table III. Occurrence of red rot in leaf scars, leaf sheaths, and internal tissues of young shoots of plant cane of seven different varieties, cultured in March and the first week of April of 1959.

Variety	No. Shoots Plated	Leaf Scar		Leaf Sheath		Internal Tissue	
		Number Plated	Per cent Red rot	Number Plated	Per cent Red rot	Number Plated	Per cent Red rot
C.P. 44-101	143	248	20.6	324	25.3	440	2.3
N.Co. 310	98	165	19.4	225	39.2	241.	2.5
C.P. 36-13	32	69	18.8	87	31.0	97	4.1
C.P. 36-105	15	45	24.4	50	28.0	68	0.0
C.P. 807	9	—	—	33	15.2	20	0.0
C.P. 34-120	3	—	—	7	0.0	3	0.0
P.O.J. 234	2	—	—	8	12.5	3	0.0

Table IV. Occurrence of the red rot fungus in leaf scars, leaf sheaths, and internal tissues of young shoots of stubble cane of five different varieties in 1957.

Location	Variety	Date of Planting	No. Shoots Planted	Leaf Scar		Leaf Sheath		Internal Tissue	
				Number Plated	Per cent Red rot	Number Plated	Per cent Red rot	Number Plated	Per cent Red rot
Caire & Graugnard Plantation	C.P. 44-101	March 21	47	93	23.7	136	43.4	154	3.2
L.S.U. Expt. Sta.	Co. 290	March 31	16	48	29.2	48	39.6	80	0.0
L.S.U. Expt. Sta.	C.P. 44-101	April 6	20	60	25.0	60	13.3	100	0.0
L.S.U. Expt. Sta.	C.P. 36-105	April 14	26	78	17.9	78	37.2	130	1.5
L.S.U. Expt. Sta.	Co. 281	April 20	16	48	25.0	58	37.9	80	0.0
Delphine Plantation	C.P. 44-101	April 28	20	45	13.3	65	7.7	89	0.0
Burley Plantation	C.P. 29-116	April 28	9	27	0.0	27	11.1	45	0.0

variety C.P. 44-101. The shoots of the variety C.P. 29-116 gave no red rot fungus from leaf scars, though 11.1 per cent of the isolations gave red rot from leaf sheath tissues. The percentage of red rot from internal tissues was 3.2 from C.P. 44-101 and only 1.5 from C.P. 36-13.

Isolations were made from 63 shoots in the month of March, 1957. The average percentage of red rot from leaf scars was 26.5 and from leaf sheaths 41.5. Isolations from 91 shoots during April gave an average percentage of red rot of 16.2 from leaf scars and 21.4 from leaf sheaths.

Table V summarizes isolation results obtained from 1957 to 1959. The percentage of red rot in leaf scars and leaf sheaths of shoots of plant cane was about the same in the years 1957 and 1959 and there was no significant difference in the numbers of isolations from plant and stubble cane. Shoots of 1957 plant cane gave 29.7 per cent red rot from leaf scars. The lowest was in 1958 plant cane which was 18.0 per cent. The highest percentage of red rot in leaf sheaths was 31.2 from 1959 plant cane and lowest, 23.3 from 1958 plant cane. The shoots of 1958 plant cane gave the lowest percentage of red rot, from both leaf scars and leaf sheaths. The internal tissues of shoots of 1958 and 1959 gave 2.06 per cent red rot in each year, the lowest being 0.69 per cent from 1957 plant cane shoots. The average percentage of red rot in all three years from leaf scars, leaf sheaths and internal tissues was 23.1, 28.0 and 1.4, respectively.

Red Rot Isolations from the Leaf Whorl of a Young Shoot.

This study was an attempt to determine whether or not the red rot

Table V. Occurrence of red rot in leaf scars, leaf sheaths, and internal tissues of the young shoots plated during 1957-1959 (Summary Table).

Year and Type of Cane	No. Shoots Plated	Leaf Scar			Leaf Sheath			Internal Tissue		
		Number Plated	Number Red rot	Per cent Red rot	Number Plated	Number Red rot	Per cent Red rot	Number Plated	Number Red rot	Per cent Red rot
1957 (Plant cane)	219	663	197	29.7	799	219	27.4	1433	10	0.69
1957 (Stubble cane)	154	399	83	20.7	472	145	30.7	678	7	1.03
1958 (Plant cane)	218	397	72	18.4	662	154	23.3	824	17	2.06
1959 (Stubble cane)	302	527	107	20.3	734	229	31.2	872	18	2.06
Total	893	1986	459		2667	747		3807	52	
Average				23.1			28.0			1.4

fungus infects laterally, from outer to the inner leaf sheaths in the young shoots. The leaf sheaths of the shoots are in a whorl and are wrapped around one another. The outer, dead leaf scales or sheaths were removed. Isolations were made from inner, living sheaths. Shoots which were about 4 to 10 inches in height were studied. The results are summarized in Table VI.

The first leaf sheath gave the highest percentage of the red rot fungus, followed by the second, third and fourth leaves. From the fifth leaf no isolates were obtained. The fungus was isolated from the fourth leaf in varieties C.P. 44-101 and Co. 290. N.Co. 310 gave red rot from the third leaf, and C.P. 36-105 only from the first leaf. No red rot was recovered from C.P. 36-13. These studies suggest the possibility of lateral spread in a young shoot as a result of infecting the leaf sheath at right angles to the main axis of the shoot, from outer to the inner leaves.

Isolations from Underground Buds of Stubble Pieces and Lateral Shoots.

Isolations were made from buds and bud scales only. The results in Table VII show that both buds and bud scales were infected, giving an average of 22.3 and 26.3 per cent red rot respectively. When buds, including scales, were cultured, the average per cent of red rot recovery was 19.1. Ungerminated buds of the seed pieces, which were not dead, were also cultured. The average percentage of red rot from these buds was 14.8 and from bud scales cultured separately, 38.7.

Isolations of Red Rot from Stubble Pieces of August Planted Cane.

Red rot was isolated from the leaf scars and internal tissues of these stubble pieces. The results are given in Table VIII. The variety

Table VI. Occurrence of red rot in leaf sheaths of young, field-grown shoots from 4 to 10 inches in height.

Variety	Number Shoots Plated	No. Pieces of Leaf Sheath Plated	Fungus Recovery From									
			1st. Leaf		2nd. Leaf		3rd. Leaf		4th. Leaf		5th. Leaf	
			Number Red rot	Per cent	Number Red rot	Per cent	Number Red rot	Per cent	Number Red rot	Per cent	Number Red rot	Per cent
C.P. 44-101	55	510	72	14.1	19	3.7	10	1.9	3	0.6	0	0.0
Co. 290	25	130	32	24.6	4	3.1	2	1.5	1	0.8	0	0.0
N.Co. 310	12	36	5	13.9	3	8.3	1	2.8	0	0.0	0	0.0
C.P. 36-105	16	64	8	12.5	0	0.0	0	0.0	0	0.0	0	0.0
C.P. 36-13	9	36	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Table VII. Occurrence of red rot in buds and bud scales of underground portions of fall-grown shoots of plant cane and also from ungerminated buds of seed pieces of different varieties, collected from different locations.

Variety	Buds From Young Shoots						Ungerminated buds of Seed Pieces			
	Bud (Scales Removed)		Bud Scales		Bud (Including Scales)		Bud (Scales Removed)		Bud Scales	
	Number Plated	Per cent Red rot	Number Plated	Per cent Red rot	Number Plated	Per cent Red Rot	Number Plated	Per cent Red rot	Number Plated	Per cent Red rot
C.P. 44-101	56	21.4	66	22.7	161	21.1	36	27.8	19	57.9
N.Co. 310	63	25.4	101	19.8	5	0.0	4	0.0	21	33.3
C.P. 36-13	10	20.0	11	36.4	5	0.0	—	—	—	—
C.P. 36-105	1	0.0	2	0.0	5	40.0	12	16.7	12	25.0
C.P. 43-47	—	—	—	—	14	0.0	—	—	—	—
C.P. 47-193	—	—	—	—	15	53.3	—	—	—	—

Table VIII. Occurrence of red rot in stubble pieces of August Planted cane; isolations from leaf scars and internal stalk tissues.

Variety	No. Stubble Plated	Leaf Scars		Internal Tissues	
		No. Pieces Plated	Per cent Red Rot	No. Pieces Plated	Per cent Red Rot
C.P. 44-101	208	869	22.7	248	29.8
C.P. 43-47	13	35	42.9	39	23.1
C.P. 47-193	12	36	0.0	36	0.0
C.P. 48-103	10	30	0.0	30	0.0
N.Co. 310	6	18	0.0	--	--
Co. 290	43	143	11.1	107	0.9

C.P. 43-47 gave 42.9 per cent red rot from leaf scars, C.P. 44-101 gave 22.7 per cent and Co. 290 only 11.1 per cent. No red rot was isolated from leaf scars of other varieties. Internal tissues of these stubble pieces gave 29.8, 23.1, and 0.9 per cent red rot in the varieties C.P. 44-101, C.P. 43-47 and Co. 290, respectively. These results show that the red rot fungus was established in these young stubble pieces, suggesting the possibility that infection occurred from the old seed pieces.

Red Rot in Sugarcane Roots.

Isolations were made from roots of young shoots to determine whether or not red rot might infect the young plants through roots. Only 2 roots out of 132 plated of the variety C.P. 44-101 gave red rot. Three out of 3 roots of C.P. 36-105 gave red rot. Out of a total of 455 roots, only 5 gave red rot fungus. The results suggest that roots are not important as a source of red rot infection for young shoots.

Occurrence of Red Rot in Young Shoots Grown From Field Inoculated and Non-inoculated Seed Pieces Planted in the Greenhouse

Previous results have shown that young shoots of plant as well as stubble cane growing in the field became infected with red rot. This was demonstrated by isolation of the fungus from sterilized portions of shoots, including leaf scars, leaf sheaths, and internal tissues. Isolations made from buds and bud scales of shoots, which were underground also gave the red rot fungus. The fungus was also recovered from leaf scars and internal tissues of underground stubble pieces of August planted cane, the shoots of which were killed during the winter.

Table IX. Isolation of red rot from roots of young shoots of different varieties of plant cane, collected from different locations.

Location	Variety	Number of Roots Plated	Number Red Rot	Per cent Red rot
L.S.U. Expt. Sta.	Co. 290	44	0	0.0
Young's Industries Plantation	Co. 290	45	0	0.0
L.S.U. Expt. Sta.	C.P. 44-101	32	0	0.0
Burley Plantation	C.P. 44-101	13	1	7.7
Burley Plantation	N.Co. 310	10	0	0.0
John Tregre Plantation	C.P. 44-101	100	1	1.0
Richard Glynn Plantation	C.P. 44-101	15	0	0.0
L.S.U. Expt. Sta.	C.P. 29-120	48	0	0.0
L.S.U. Expt. Sta.	C.P. 34-120	76	0	0.0
L.S.U. Expt. Sta.	C.P. 36-105	3	3	100.0
L.S.U. Expt. Sta.	C.P. 47-193	30	0	0.0
L.S.U. Expt. Sta.	C.P. 52-103	33	0	0.0
L.S.U. Expt. Sta.	C.P. 53-1	6	0	0.0

The studies reportedin this section were made in order to determine whether or not the fungus moves from infected stalks, used as seed pieces, into young shoots under greenhouse conditions.

Results, shown in Table X, are that the red rot isolations from leaf scars, leaf sheaths, growing points, and internal rolled leaves from shoots of inoculated seed pieces of the variety C.P. 44-101 were 6.3, 2.2, 3.0, and 0.7 per cent respectively (Plate IV-1). From shoots of non-inoculated seed pieces of the same variety red rot percentages were 2.7, 2.9, 0.0, and 0.4, respectively. Leaf scars, leaf sheaths, growing points and internal rolled leaves from the shoots of inoculated seed pieces of the variety Co. 290 gave 29.4, 7.8, 4.8, and 1.3 per cent red rot respectively (Plate IV-2). Shoots of non-inoculated seed pieces of the variety Co. 290 gave 5.9 per cent from leaf scars, 8.8 per cent from leaf sheaths, 2.1 per cent from growing point and none from internal rolled leaves. From these results, it may be seen that leaf scars, growing points, and internal rolled leaves from the shoots of inoculated seed pieces in both varieties, Co. 290 and C.P. 44-101, gave higher percentages of the red rot fungus than did similar tissues from the shoots of non-inoculated seed pieces. However, leaf sheath tissues from shoots of inoculated and non-inoculated seed pieces did not show a significant difference in red rot recovery. It will be observed that the variety Co. 290 yielded more red rot from all shoot tissues cultured except internal rolled leaves than did C.P. 44-101 in both inoculated and non-inoculated seed pieces. Isolations from leaf scars of old seed pieces also gave the red rot fungus. The results here suggest that shoots may become infected from the red rot fungus in the old seed pieces. Just how the fungus

Table X. Isolation of red rot from leaf scars of seed pieces, and from leaf scar, leaf sheaths, growing points, and internal rolled leaves of young shoots grown in the greenhouse from these seed pieces.

Variety	Treatment	Seed Piece Isolations: Leaf Scar		Shoot Isolations: Leaf Scar		Leaf Sheath		Growing Point		Internal Rolled Leaves	
		Number Plated	Per cent Red Rot	Number Plated	Per cent Red Rot	Number Plated	Per cent Red Rot	Number Plated	Per cent Red Rot	Number Plated	Per cent Red Rot
C.P. 44-101	Inoculated	72	12.5	32	6.3	138	2.2	66	3.0	144	0.7
"	Non-Inoculated	75	2.7	74	2.7	305	2.9	99	0.0	284	0.4
Co. 290	Inoculated	72	8.3	34	29.4	154	7.8	62	4.8	141	1.3
"	Non-Inoculated	72	4.2	51	5.9	272	8.8	97	2.1	259	0.0

moves from diseased seed cane into new plants is not known. Observations would suggest, however, that the fungus moves from the old mother piece infected bud scales into contiguous healthy outer scales of the emerging young bud, and then the leaf sheaths of the young roots.

In Table XI it will be seen that more shoots develop red rot in cane grown from inoculated seed pieces than in that from non-inoculated seed cane. The shoots from both inoculated and non-inoculated seed pieces of the variety Co. 290 gave a higher percentage of red rot infection than did the shoots from inoculated and non-inoculated seed pieces of the variety C.P. 44-101.

Study of Penetration of the Red Rot Fungus Through the Upper Surface of Midrib Pieces Inoculated in Moist Chambers

It has been reported that red rot infection does not take place on the upper side of the midribs because of the presence of a waxy coating and an underlying layer of 4 to 5 cells of sclerenchyma tissues. (Plate XV). That may be the reason that no satisfactory symptoms were produced. In order to further study the possible infection through uninjured upper epidermal layers of midribs, the following procedures were used. Leaves from the field, as well as from the greenhouse, showing no visible symptoms or injury were selected. Pieces were cut from these leaves, sterilized, and the cut ends were waxed before inoculations. They were inoculated in moist chambers by placing spore suspensions on both injured and uninjured leaf surfaces. Isolations were made after one, two, three, four and five days. Results are given in Table XII.

After one day of inoculation the recovery from injured leaves was 86.6 and 73.3 per cent in Co. 290 and C.P. 44-101 respectively, and it

Table XI. Occurrence of red rot in shoots grown in the greenhouse from inoculated and non-inoculated seed cane.

Variety	Treatment	No. Shoots Plated	Shoots Giving the Fungus Number	Shoots Giving the Fungus Per cent
C.P. 44-101	Inoculated	32	12	37.5
	Non-inoculated	66	9	13.6
Co. 290	Inoculated	32	16	50.0
	Non-inoculated	63	17	26.9

XII. Red rot recovery from field grown midribs, inoculated in moist chambers and isolations made after one, four and five days.

Treatment	Isolations From Midrib After One Day: Number Pieces Plated	One Day: Per cent Red rot	Four Days: Number Pieces Plated	Four Days: Per cent Red rot	Five Days: Number Pieces Plated	Five Days: Per cent Red rot
Co. 290						
Injured-inoculated*	30	86.6	25	100.0	30	100.0
Uninjured-inoculated	30	53.3	26	92.3	30	93.3
Injured-check	30	3.3	15	6.6	15	13.2
Uninjured-check	20	6.6	10	10.0	15	6.6
C.P. 44-101						
Injured-inoculated*	30	73.3	25	96.0	30	100.0
Uninjured-inoculated	30	70.0	25	84.0	35	92.0
Injured-check	30	3.3	15	13.3	15	6.6
Uninjured-check	30	10.0	10	10.0	15	6.6

*Injury by needle punctures.

was 100 per cent after 4 days and 5 days in both varieties. The recovery, after one day, from uninjured, inoculated midrivs was 53.3 per cent in Co. 290 and 73.3 in C.P. 44-101. After 4 and 5 days of inoculation the average percentage recovery was 92.8 from uninjured Co. 290 and 88 from C.P. 44-101. The non-inoculated checks of both varieties also gave the red rot fungus. This was due most probably to the light field infection of leaves during the last summer months, though they showed no symptoms when collected. In further studies, only the leaves of greenhouse grown plants were used.

In Table XIII, results are presented of an experiment in which leaves from greenhouse grown plants only were used for inoculation. The isolations were made after one, two, three, four and five days in the moist chambers. In this experiment, no red rot fungus was isolated from non-inoculated checks. Recovery of red rot from the injured, inoculated midribs after two to five days was 100 per cent in both Co. 290 and C.P. 44-101 varieties. One day after inoculation of injured leaves, 62.9 and 65.7 per cent infection occurred in C.P. 44-101 and Co. 290 respectively. From uninjured, inoculated midribs, the recovery of the fungus after one day was 42.8 per cent from Co. 290 and 28.6 per cent from C.P. 44-101. The average percentage of red rot recovery in Co. 290 and C.P. 44-101 varieties from the uninjured, inoculated midribs after one day was 35.7 per cent; after two days, 73.8 per cent; after three days 79.1 per cent; and after four and five days 92.8 per cent. These results indicate that the red rot fungus penetrates the uninjured midribs from the upper surface, producing incipient infections without typical red rot lesions (Plate V).

Table XIII. Red rot recovery from greenhouse grown leaf midribs, inoculated in moist chambers, and isolations made after one, two, three, four and five days.

Treatment	Isolations From Midrib After									
	One Day		Two Days		Three Days		Four Days		Five Days	
	No. Pieces Plated	Per cent Red rot	No. Pieces Plated	Per cent Red rot	No. Pieces Plated	Per cent Red rot	No. Pieces Plated	Per cent Red rot	No. Pieces Plated	Per cent Red rot
Co. 290										
Injured-inoculated*	35	62.9	70	100.0	135	100.0	35	100.0	95	100.0
Uninjured-inoculated	35	42.8	65	64.6	130	75.4	35	94.2	75	92.1
Injured-check	35	0.0	60	0.0	65	0.0	15	0.0	40	0.0
Uninjured-check	35	0.0	50	0.0	60	0.0	15	0.0	40	0.0
C.P. 44-101										
Injured-inoculated	35	65.7	65	100.0	135	100.0	45	100.0	95	100.0
Uninjured-inoculated	35	28.6	65	83.0	140	82.8	35	91.4	75	93.3
Injured-check	35	0.0	65	0.0	60	0.0	20	0.0	40	0.0
Uninjured-check	35	0.0	55	0.0	65	0.0	20	0.0	40	0.0

*Injured by needle punctures.

Hand sections from the uninjured inoculated midribs were made. Microscopic examination of these sections showed that the spores germinated by producing appressoria. These appressoria were observed c cemented to the surface of the midrib. Infection threads from these were seen, in some cases, to enter through the waxy coating and into the epidermal cells.

The effect of length of time of surface sterilization upon the recovery of red rot from uninjured inoculated midribs was also studied and the results are presented in Table XIV. The midrib pieces of the variety C.P. 44-101 were used. Inoculations were made on uninjured upper surfaces of the midribs in moist chambers. Reisolations from these were made 4 days later after sterilizing in a solution of 1:1000 mercuric chloride in 50 per cent alcohol for different lengths of time. Time of sterilization was 5, 10, 15, 20, 30, 40, 50, 60 and 120 minutes. The percentage recovery of the red rot fungus after 5 minutes was 82.5, after 10 minutes 55.7, after 15 minutes 37.5, after 20 minutes 26.5, after 30 minutes 14.3, after 40 minutes 16.3, after 50 minutes 8.7, after one hour 7.8 and after two hours 0.7. These results indicate that there is a definite effect of time of surface sterilization upon the recovery of red rot from the uninjured inoculated midribs.

Study of Penetration of the Red Rot Fungus Through the Upper and Lower Surfaces of Leaf Midribs on Greenhouse Plants

In laboratory studies, the red rot fungus was found to infect the upper surface of leaf midribs in uninjured condition which was demonstrated by the reisolation of the fungus from such tissues after sterilization for two hours. The data reported here resulted from a study of the infection

Table XIV. Effect of length of time of surface sterilization upon recovery of red rot from inoculated uninjured midribs in moist chambers.

Surface Sterilization				
1:1000 $HgCl_2$ in 50 Per cent Alcohol	No. Midribs Inoculated	No. Pieces Plated	No. Isolated	Per cent Red rot
5 minutes	16	80	66	82.5
10 minutes	28	140	78	55.7
15 minutes	16	80	30	37.5
20 minutes	16	80	21	26.5
30 minutes	28	140	16	14.3
40 minutes	16	80	13	16.3
50 minutes	16	80	7	8.7
1 hour	28	140	11	7.8
2 hours	28	140	1	0.7

of leaf midribs in their natural condition while on the plants. The midribs were inoculated with a spore suspension, on injured and uninjured lower and upper surfaces of midribs. Non-inoculated checks were kept for each treatment. Inoculated portions were covered by wax papers to prevent drying (Plate I). These artificial moist chambers on the leaf blades were removed after 48 hours. Development of symptoms was observed for as long as one month after which time isolations were made from midribs. The results, shown in Table XV, demonstrated that in varieties Co. 290 and C.P. 44-101, visible midrib symptoms developed on all injured leaves, regardless of whether inoculations were on upper or lower surfaces. The symptoms appeared in 48 hours and the disease spread from the points of injury to the uninjured midrib portions. From observations made during disease development, the spread was more rapid towards the tip of leaf from the site of inoculation (Plate VI). In uninjured leaves, a few lesions occurred on some leaves. These were pin-point type lesions which were observable after 15 or more days. Leaves showing such symptoms, were in a state of lowered vitality. In non-inoculated checks no symptoms were observed.

The inoculated leaf midribs were removed from the plants after one month and isolations were made. The results are presented in Table XVI. From the injured, inoculated leaves of both upper and lower surfaces in varieties Co. 290 and C.P. 44-101, the recovery of red rot was 100 per cent. In the case of uninjured upper surfaces, the percentages of isolation were 55.3 for Co. 290 and 61.9 for C.P. 44-101. Isolations from uninjured, inoculated lower surfaces of midribs were 66.4 per cent for Co. 290 and 75.7 per cent for C.P. 44-101 leaves. The recovery of red rot was higher from the uninjured, inoculated lower surfaces of midribs

Table XV. Red rot development on midribs, inoculated with spore suspensions in the greenhouse*

Leaf Surface Treatment	Co. 290			C.P. 44-101		
	No. of Leaf Midribs	Midribs Showing Symptoms		No. of Leaf Midribs	Midribs Showing Symptoms	
		No.	Per cent		No.	Per cent
Inoculated						
Upper-injured	39	39	100.0	40	40	100.0
Lower-injured	38	38	100.0	37	37	100.0
Upper-uninjured	60	5	8.3**	45	12	26.7**
Lower-uninjured	56	4	7.1**	46	13	28.3**
Non-inoculated						
Upper-injured	16	0	0.0	18	0	0.0
Lower-injured	18	0	0.0	19	0	0.0
Upper-uninjured	18	0	0.0	19	0	0.0
Lower-uninjured	18	0	0.0	19	0	0.0

*See Plate 1, showing artificial moist chambers.
**Pin point lesions assumed to be incipient infections.

Table XVI. Recovery of red rot from midribs, inoculated on plants of Co. 290 and C.P. 44-101; isolations one month later.

Leaf Surface Treatment	Co. 290			C.P. 44-101		
	Number Pieces Plated	Number Red rot	Per cent Red rot	Number Pieces Plated	Number Red Rot	Per cent Red rot
Inoculated						
Upper-injured	234	234	100.0	240	240	100.0
Lower-injured	228	228	100.0	222	219	98.6
Upper-uninjured	360	198	55.3	270	167	61.9
Lower-uninjured	336	223	66.4	276	209	75.7
Non-inoculated						
Upper-injured	80	0	0.0	90	0	0.0
Lower-injured	90	0	0.0	95	0	0.0
Upper-uninjured	90	0	0.0	95	0	0.0
Lower-uninjured	90	0	0.0	95	0	0.0

than from upper surfaces. The average percentage of recovery from Co. 290 leaves in the uninjured condition was 60.8 per cent while in C.P. 44-101 it was 68.8 per cent. The non-inoculated leaves in all treatments did not give the red rot fungus. These results demonstrate that the red rot fungus penetrates the healthy leaf midribs, causing pin-point lesions. This incipient infection does not produce typical symptoms of the disease.

Inoculation of Greenhouse Plants by Spraying.

Greenhouse-grown plants when about 3 to 3-1/2 feet in height were inoculated by spraying with a spore suspension. Plants were inoculated in both injured and uninjured conditions. The injury was made by pricking the upper surface of the leaf midribs with a fine needle. Non-inoculated check plants were included in the experiment. After inoculation, plants were transferred to a greenhouse moist chamber and kept for 48 hours. Development of symptoms in these plants was observed for two months. The results are given in Table XVII. On the injured plants, all the leaves showed symptoms in both varieties studied. These lesions later coalesced and covered the entire midrib. The leaves of the plants not injured showed no symptoms. Non-inoculated checks were free of disease.

Development and Spread of Red Rot From Inoculated Leaf Sheaths to Midribs, in the Greenhouse

Studies reported earlier in this paper have shown that leaf sheath tissues of the young underground shoots are infected with red rot. Later in the season leaf sheaths of the lower leaves become infected.

Table XVII. Appearance of red rot symptoms in greenhouse-grown plants inoculated by spraying spore suspensions on injured* and uninjured plants.

Variety	Injured			Not Injured		
	No. of Plants	No. of Leaves	No. Leaves Showing Visible Symptoms	No. of Plants	No. of Leaves	No. Leaves Showing Visible Symptoms
Inoculated						
C.P. 44-101	11	45	45	13	59	0
Co. 290	9	41	41	10	53	0
Non-inoculated						
C.P. 44-101	2	10	0	2	11	0
Co. 290	2	10	0	2	12	0

*Injury made by pricking leaf midrib with a fine needle.

These studies were undertaken to determine whether or not there is a relationship between leaf sheath infection and the development of the disease in the leaf midribs. These studies were carried out in the greenhouse. Leaves were inoculated by placing spore suspensions behind the leaf sheaths when they started pulling away from the stalks. The leaves inoculated in both injured and uninjured conditions. The leaf sheaths were injured on the inside by pricking with a needle.

Symptoms in uninjured leaf sheaths developed within 48 hours. The disease developed on the midribs within 6 days with more lesions appearing thereafter for 20 days or more in the non-injured leaves (Plates VII, VIII, IX, X). In injured leaf sheaths, symptoms were observed within 24 hours and separated lesions appeared within 48 hours on the under surface of the midrib. The development of red rot lesions on midribs of injured leaves within 48 hours after inoculation suggests that the injury permitted inoculum to enter broken vascular bundles, moving upward into the blade midrib where spores lodged and produced dispersed lesions. The delayed development of lesions on midribs of uninjured leaves suggests that spores may have been produced within diseased tissues of inoculated leaf sheaths and gained entrance to vessels of vascular bundles, moving up to leaf midribs where lesions were produced, six days or more after inoculation.

The development of red rot symptoms on leaf midribs was studied in four varieties of sugarcane after leaf sheath inoculations. Both susceptible and resistant varieties were included in these experiments. The results are presented in Table XVIII. It will be noted that red rot developed on midribs of more leaves when leaf sheaths were injured than when leaf sheaths were not injured, regardless of susceptibility of

Table XVIII. Development of red rot lesions on blade midribs after leaf sheath inoculations in the greenhouse.

Variety	Sheath Injured			Sheath Not Injured		
	No. of Leaves	No. Showing Blade Symptoms	Per cent	No. of Leaves	No. Showing Blade Symptoms	Per cent
Inoculated						
C.P. 44-101	186	167	89.8	458	166	36.2
Co. 290	114	99	86.8	378	148	33.1
C.P. 36-105	10	8	80.0	43	4	9.3
C.P. 36-13	5	3	60.0	5	0	0.0
Non-inoculated						
C.P. 44-101	33	0	0.0	224	0	0.0
Co. 290	19	0	0.0	165	0	0.0
C.P. 36-105	8	0	0.0	18	0	0.0
C.P. 36-13	5	0	0.0	5	0	0.0

variety. Symptoms developed on all inoculated leaf sheaths, injured and uninjured. When leaf sheaths were injured, the leaves showing midrib symptoms was 89.8 per cent in the variety C.P. 44-101 and 86.8 per cent in Co. 290. But where leaf sheaths were not injured, the leaves showing m midrib symptoms were 36.2 and 33.1 per cent in the varieties C.P. 44-101 and Co. 290 respectively. Eighty per cent of the leaves showed midrib symptoms, when injured, in the variety C.P. 36-105, whereas, only 9.3 per cent in uninjured leaveas developed symptoms. Though the number of leaves inoculated, of the variety C.P. 36-13, was not very high, 60 per cent of the leaves showed midrib symptoms when injured and none in uninjured leaves. The leaves kept as checks were free of midrib as well as sheath symptoms. Close observations made during these studies revealed that the non-inoculated leaves on the plants, containing inoculated leaves, did not develop red rot symptoms on the midribs.

Isolations from the sheath-inoculated leaves were made and are presented in Table XIX. The cultures were made from the inoculated leaf sheath, from midrib lesions, from portions of the midrib between the lesions, from blade (lamina) lesions, and from symptomless midribs. The number of leaves plated was 255 for C.P. 44-101 and 145 for Co. 290. The recovery of the red rot fungus from leaf sheath tissue was high, but not 100 per cent which suggests that not all the reddened tissues from different locations in the leaf sheath contained the fungus. Midrib lesions of the variety C.P. 44-101 gave 74.4 per cent red rot and Co. 290 gave 53.6 per cent. This also suggests that the reddening of the midrib tissues does not necessarily mean the presence of red rot fungus. Some of the reddening might have been due to the presence of a gummy-like

Table XIX. Isolation of red rot from different tissues of leaves which had been inoculated back of leaf sheaths in the greenhouse.

Source of Tissue Isolations	Plated		Giving the Fungus	
	No. of Leaves	No. of Pieces	Number	Per cent
C.P. 44-101				
Leaf sheath lesions	255	344	297	86.6
Midrib lesions		539	401	74.4
Between lesions on midrib		178	69	38.8
Blade (lamina) lesions		68	20	29.4
Midribs with no visible symptoms		2,838	52	1.8
Co. 290				
Leaf sheath lesions	145	159	112	70.4
Midrib lesions		263	141	53.6
Between lesions on midribs		103	84	81.6
Blade (lamina) lesions		45	21	46.7
Midribs with no visible symptoms		1,787	52	2.9

material which is produced within the host tissues. Or it could possibly have been caused by some toxic-like substances which might have been produced by the fungus working in advance of the causal organism. Isolations made from between the lesions of the variety C.P. 44-101 gave 38.8 per cent red rot (Plate X). Similar isolations from Co. 290 gave 81.6 per cent. A high percentage of red rot was recovered from the variety Co. 290 from between the lesions. Since midrib lesions were not continuous and the fungus was not always recovered from between the lesions, this suggests that the dispersed midrib lesions were not the result of a continuous mycelium passing through the midrib tissues from one lesion to another but they may be the result of spore migration through vessels. The source of such inoculum could either be from external through leaf sheath prickings or from the spores which might have been produced within the host tissues. Lesions on the blade (lamina) also gave red rot cultures.

Study of Host-parasite Relationships.

Hand sections of midrib and leaf sheath tissues were made from the leaves whose leaf sheaths had been inoculated in the greenhouse and where midrib symptoms had developed. Sections through red rot lesions were made in order to study the production of spores within the host tissues. Sections of the midrib were cut through young lesions, old lesions not showing the presence of acervuli on the surface, lesions which had produced acervuli, and from between lesions. These sections were studied by microscopic examinations.

Sections of the portions of midrib between two lesions showed no mycelium, though such sections usually showed a discoloration of the

vessels. Sometimes only one vessel in a vascular bundle showed reddening. When sections were made through a lesion, mycelium was often found in the vessels. The mycelium from the parenchyma passes through the cell walls into the adjoining vessels. In the early stages of the lesion, the mycelium is small in diameter within the host cells (Plate XI). Later, as the lesions become older, the mycelium inside the host cells becomes larger in diameter. A gummy-like material, whose nature is not known, fills the host cells in advance of the fungus. The mycelium was observed passing from one cell to another through the pits of the cell walls. The pit can be seen clearly.

During these studies, the production of atypical spores was also observed in the parenchyma cells (Plate XII) and inside of large vessels (Plate XIII). Production of more typical spores was also observed on old mycelium in parenchyma cells (Plate XIV). Sections of midribs which had already produced acervuli and setae on the upper surface showed how the fungus pushes itself out through 4 or 5 layers of thick sclerenchyma cells which lie beneath the upper epidermis. The acervuli containing conidiophores with conidia and the setae are shown in Plate XV.

Spread of Red Rot Spores Through the Cut Ends of Detached Sugarcane Leaves

The purpose of this experiment was to study the migration of red rot spores from leaf sheaths to the blade through the ligular region in detached leaves in comparison with leaves whose leaf sheaths were removed. Healthy sugarcane leaves were removed from greenhouse plants and brought to the laboratory. The leaves were cut under water to avoid

the possibility of air vacuum in the vessels. Leaves were then placed in a spore suspension for four hours andthen removed to flasks containing water. Checks, non-inoculated, were kept in water only.

Red rot lesions first appeared on the submerged portions of leaves which was placed in the inoculum. After 36 hours, lesions appeared on the lower surface of non-submerged midribs having no leaf sheaths attached and after 48 hours in leaves with leaf sheaths attached. No upper-surface midrib symptoms were observed for 3 days and in some cases 4 days. The final measurement of red rot spread in midrib was taken after 7 days. This indicated the length of migration of spores through the vessels of detached leaves. The results are recorded in Table XX and show that the average length of spread in leaf midribs without leaf sheaths after 7 days was 31.8 cms. in variety C.P. 44-101 and 25.6 cms. in Co. 290. In leaves with sheaths attached, the average length in centimeters was 11.9, in leaves of C.P. 44-101 and 9.2 in leaves of Co. 290. This also included the migration through 10 cms. of leaf sheath tissues. Leaves kept in water were free from any disease symptoms. In both cases, the spread in C.P. 44-101 leaves was higher than in Co. 290. It was observed that the Co. 290 leaves wilted faster than C.P. 44-101 leaves when put in the red rot inoculum Checks, in water only, remained healthy. These results show that the red rot spores are carried from leaf sheaths to leaf midribs through the ligular region without any difficulty in the varieties studied.

Table XX. Spread of red rot in detached sugarcane leaves after dipping cut ends for four hours in a spore suspension.

Treatment	Leaves	Leaf Sheath Length in cm.	Ave. Length of Spread of Red Rot in Midribs After 7 Days in cm.
Co. 290			
Leaves with leaf sheaths in spore suspension	11	10.0	9.2
Leaf blades only in spore suspension	10	—	25.6
Leaves with leaf sheaths in water (check)	7	10.0	0.0
Leaves without leaf sheaths in water (check)	7	—	0.0
C.P. 44-101			
Leaves with leaf sheaths in spore suspension	16	10.0	11.9
Leaf blades only in spore suspension	25	—	31.8
Leaves with leaf sheath in water (check)	10	10.0	0.0
Leaves without leaf sheath in water (check)	12	—	0.0

Infection and Development of Red Rot in the Leaves of Sugarcane Under Field Conditions

Red rot midrib symptoms do not appear on the leaves until July or August in Louisiana. As has been mentioned previously, red rot was recovered from the uninjured, inoculated midribs, even though there were no visible symptoms on these leaves. A latent type of infection of sugarcane stalks by the red rot fungus has been reported to be quite prevalent in Louisiana. It was thought that such might be the case with the leaves of cane in the field and that infection took place earlier but the fungus remains dormant until environmental conditions become favorable for production of midrib symptoms. An attempt was made to determine whether or not there is a latent infection in leaf midribs. Isolations were made from healthy leaves, from leaves with lesions, and from between lesions. The presence of red rot infection in the leaf sheaths was also studied.

The leaves which showed no visible symptoms, but only a reddening of the leaf sheaths, were collected from the field. Six varieties of cane were studied, which included both susceptible and resistant varieties. After washing and sterilizing, leaf sheaths were placed in laboratory moist chambers. Five days later, they were examined under the microscope for the presence of red rot conidia. The results of this experiment are summarized in Table XXI, and show that the highest percentage of red rot infection was 45.0 in the variety C.P. 29-320, which is moderately susceptible. The second highest was 31.0 per cent in Co. 290, a susceptible variety; the third highest was 22.7 per cent in C.P. 34-120, also a moderately susceptible variety. The resistant variety

Table XXI. Occurrence of the conidial stage of red rot fungus inside of the reddened leaf sheaths collected from different varieties of sugarcane in the field.

Variety	Type of Resistance	No. of Leaf Sheaths Examined	Leaf Sheaths Found Infected	
			Number	Per cent
Co. 290	Susceptible	29	9	31.0
C.P. 29-320	Moderately susceptible	20	9	45.0
C.P. 34-120	Moderately susceptible	22	5	22.7
C.P. 36-105	Resistant	32	3	9.7
C.P. 44-101	Resistant	36	7	19.4
C.P. 52-68	Resistant	33	6	18.2

C. P. 36-105 was found to be the lowest, 9.7 per cent of leaf sheath infection. The other two resistant varieties, C.P. 44-101 and C.P. 52-68 were found to have 19.4 and 18.2 per cent infection of leaf sheaths respectively. It cannot be concluded from these results that there is a varietal difference to the leaf sheath infection of the six varieties studied, though a resistant variety C.P. 36-105 showed a lower percentage of leaf sheath infection than did the other varieties.

Occurrence of Red Rot in Apparently Healthy Leaves.

Symptomless, leaves, showing no reddening were collected from several varieties of cane in the field and at different times of the year. Isolations were made by random selection of five to six pieces of midrib from each leaf. These pieces were sterilized for 5 to 7 minutes in a solution of 1:1000 mercuric chloride in 50 per cent alcohol before plating. The results are presented in Table XXII, and show that a total 157 leaves were used for isolation on April 26 and June 2, or a total of 1,024 pieces were plated. None gave red rot. On June 19, 1959, 155 leaves of five different varieties were used with a total of 1,006 pieces being plated. Of this number only 7 pieces gave the red rot fungus, which is 0.7 per cent. During the month of July, 71 leaves were used, with a total of 412 pieces being plated. From these, 67 gave the fungus, which is 16.2 per cent. Out of 58 healthy leaves plated during September, 348 pieces were cultured and the red rot fungus was recovered from 103 of these, or 29.6 per cent. These results indicate that the red rot fungus occurs in apparently healthy leaves and suggests incipient infection, which might develop into visible lesions. Later in the season a higher percentage of red rot was recovered from apparently

Table XXII. Isolation of red rot fungus from apparently healthy leaves collected from different varieties of sugarcane at different dates in the field.

Date of Plating	Variety	No. of Leaves	Number Pieces Plated	Number Red rot	Per cent Red rot	No. Other Fungi
4-26-57	C.P. 44-101	25	285	0	0.0	-
6-2-59	Co. 290	21	126	0	0.0	124
6-2-59	C.P. 44-101	46	274	0	0.0	182
6-2-59	C.P. 36-105	39	233	0	0.0	110
6-2-59	C.P. 52-68	26	106	0	0.0	21
6-19-59	Co. 290	22	132	2	1.5	120
6-19-59	C.P. 44-101	40	270	2	0.7	68
6-19-59	C.P. 36-105	39	234	1	0.4	122
6-19-59	C.P. 52-68	33	244	2	0.8	104
6-19-59	N.Co. 310	21	126	0	0.0	48
7-8-59	Co. 290	35	210	14	6.7	-
7-10-57	C.P. 44-101	11	64	0	0.0	-
7-15-57	C.P. 36-105	10	48	1	2.1	-
7-29-57	Co. 290	15	90	52	57.8	-
9-10-57	Co. 290	17	102	32	31.4	-
9-14-57	Co. 290	21	126	44	34.9	-
9-21-57	Co. 290	20	120	27	22.5	-
9-21-57	C.P. 44-101	25	150	22	14.7	-

healthy leaves, suggesting a positive correlation with the increase amount of inoculum during the summer months.

Isolation of Red Rot From Midrib Lesions.

Midrib lesions were cultured and data are summarized in Table XXIII. The leaves showing reddening of leaf midribs were collected from the field during the growing season, starting May 15. From May 15 to June 29, 1959 leaves were used for isolations and 1,002 pieces were plated. No red rot fungus was obtained. During the month of July, 82 leaves ere used and 391 pieces from these were plated. From these, 57 colonies of red rot, or 14.5 per cent were recovered. In August, from 64 leaves, 187 pieces of reddened midrivs were plated. Forty seven, or 2.5 per cent gave red rot. The results show that red rot lesions begin appearing in July. The reddening of midribs during the earlier part of the growing season is apparently not due to the red rot fungus.

Isolation From Between Lesions.

Midrib sections from between lesions were cultured in order to determine if two such separated lesions are connected directly by mycelium. Isolations were made during August and September. The results are presented in Table XXIV, and show that a high percentage of red rot recovery was not made from 100 per cent of the tissue pieces plated, it is apparent that there is no mycelial connection between some lesions and consequently it must be concluded that such lesions were due to separate infections.

Table XXIII. Isolation of red rot fungus from midrib lesions appearing in the field at different dates.

Date of Plating	Variety	No. of Leaves	Number Pieces Plated	Number Red rot	Per cent Red rot	No. Other Fungi
5-15-58	Co. 290	20	151	0	0.0	104
5-17-58	C.P. 44-101	12	60	0	0.0	15
5-27-58	Co. 290	24	76	0	0.0	0
6-2-59	---	21	127	0	0.0	112
6-19-59	---	36	267	0	0.0	82
6-20-59	---	32	127	0	0.0	109
6-24-59	---	27	120	0	0.0	99
6-29-59	---	23	92	0	0.0	71
7-5-59	---	16	86	4	4.7	77
7-8-57	Co. 290	32	161	1	0.6	-
7-10-57	C.P. 44-101	9	32	0	0.0	-
7-16-57	C.P. 36-105	9	32	0	0.0	-
7-29-57	Co. 290	16	80	52	65.0	-
8-12-58	---	22	88	14	15.9	44
8-19-58	---	42	99	33	33.3	0

Table XXIV. Isolation of red rot from symptomless areas between the lesions on midribs from the leaves collected in the field at different dates.

Date of Plating	Variety	No. of Leaves	No. Pieces Plated	Number Red rot	Per cent Red rot
8-12-57	C.P. 44-101	12	66	43	65.2
8-15-57	Co. 290	10	36	22	61.1
8-15-57	N.Co. 310	8	34	14	41.2
8-15-57	C.P. 44-101	7	28	23	82.1
8-15-57	C.P. 52-68	16	48	36	75.0
9-10-57	Co. 290	16	96	41	42.7
9-15-57	C.P. 36-105	20	80	53	63.3
9-15-57	C.P. 29-320	15	75	50	66.6
9-26-57	C.P. 34-120	19	95	63	66.3

DISCUSSION

One of the most interesting problems with the red rot disease of sugarcane, is the manner in which infection of the young plants takes place. In the literature, the direct infection of the young growing shoots from the diseased seed cane has been reported with divergent opinions. Butler (15) was the first to claim that the mycelium of the red rot fungus passed directly from the old diseased seed cuttings into the young plants. Before Butler's work, Raciborski (51) in Java reported infection of young plants from seed pieces. In Louisiana, Edgerton (29) reported that infection of the young shoots from the old seed pieces did not take place, although young plants frequently died as a result of severe infection of the planted stalks. Butler and Hafiz Khan (17) repeated Butler's experiments, and confirmed his original conclusion. Abbott (6) reported that in no instance was the red rot fungus observed spreading from diseased cuttings into the shoots arising from the buds there upon. He reported, that sometimes the cuttings were so rotted that the shoots would die before establishing their own root systems. Abbott's results (6) supported Edgerton's conclusions that direct mycelial connections between the seed cuttings and the growing shoots did not occur in Louisiana, or at least was so rare, as to be of no importance in initiating the infection in young plants.

During the present investigation, an attempt was made to demonstrate the development of the red rot fungus from the infected seed pieces to the young shoots. A latent type of bud and leaf scar infection of sugarcane stalks, used for planting in Louisiana, has been

reported by Steib (53). This latent type bud infection or dormant mycelium was thought to carry the fungus from the buds to the growing shoots. The young growing shoots from the field were used in the present studies. Isolations were made from different portions of these shoots and from the parts which were underground. When isolations were made from the leaf scars, leaf sheaths, internal tissues including growing points, and internal rolled leaves of the young shoots, the occurrence of the red rot fungus was demonstrated. The leaf sheath tissues generally gave higher percentages of red rot than did the leaf scars or the internal tissues. In a few cases, as high as 5 per cent of the internal tissues plated gave red rot. The average recovery of red rot during these studies from leaf scars, leaf sheaths and internal tissues was 23.1, 28.0 and 1.4 per cent respectively, demonstrating that the young growing plants are infected with the red rot fungus.

Sugarcane in Louisiana is planted in fields which are usually fallow plowed for one year or more. This destruction of sugarcane debris probably reduces to a minimum the possibility of soil infection in plant cane, since the literature reports that the fungus can not live more than five to six months in the soil or soil debris. The principal method of persistence of the fungus then is infected seed pieces.

Butler (16) reported that the red rot fungus, though present in the planted seed cane, spread into the young developing shoots without killing them. In the studies reported in this paper, the young shoots used for isolations were apparently healthy.

Edgerton (29) examined the young plants from diseased seed pieces

in the greenhouse andfield by splitting directly through them, and did not see any reddening passing from the diseased stalk into the young plant. Steib (54), on the other hand, reported that in healthy shoots, attached to the seed pieces kept in storage, the disease developed and the infection occurred at the base of the shoot at the point of contact with the old bud scales.

In the studies reported here, shoots attached to seed pieces were also kept in storage. The shoots were split and discoloration was found in the internal tissues. When isolations were made from these reddened internal tissues, the red rot fungus was recovered. This confirms Steib's results, that infection occurred at the base of the shoot at the point of contact with the old bud scales. This was also observed in field-collected shoots used for isolation. These observations would suggest, that under unfavorable growth conditions, the fungus from the infected bud scales of the old mother seed pieces invaded contiguous healthy outer scales of the emerging young bud and eventually the leaf sheath of the young shoot. McMartin (48) also suggested that disintigrated material surrounding the base of young plants might be a source of inoculum for young stalks. He also reported that leaf sheaths had discolored areas, which were proved to be lesions of red rot. McMartin suggested, that the fungus may grow from the infected material in the soil up the outside stem or leaf sheath.

Underground buds and bud scales of the stubble pieces and their lateral young shoots were also found infected with red rot. Isolation from buds and bud scales gave an average of 22.3 and 26.3 per cent red rot respectively. These results suggest, that not only the shoots but

their lateral buds, which would later become shoots, are also infected with red rot.

Infection of young shoots was studied under greenhouse conditions by planting field-inoculated and non-inoculated seed cane. Isolations were made from leaf scars, leaf sheaths, growing points and internal rolled leaves of developing shoots, and the average recoveries were 17.9, 5.0, 3.9 and 1.0 per cent respectively from field-inoculated seed cane, and 4.3, 5.9, 1.1 and 0.2 per cent, respectively, from the non-inoculated cane. These results demonstrated that infection in young shoots was directly proportional to the extent of infection in seed pieces.

In regard to leaf midrib infection, it has been suggested that the red rot fungus may infect directly. Butler and Hafiz Khan (17) were successful in producing the disease on uninjured midribs, but when no injuries were made, only a few inoculations were successful. They concluded that the fungus was capable of penetrating the uninjured leaves, but infection occurred much more readily in injured leaves. Abbott (6) also reported that infection of red rot will take place through apparently uninjured leaves. Edgerton and Carvajal (35) reported that when spore suspensions were sprayed on the upper surface of the midrib, satisfactory infection was not obtained, though a few lesions were observed, which did not increase in size.

During the present studies, leaf midribs inoculated with injury in moist chambers or on greenhouse plants, developed red rot which later spread to the whole blade. When inoculations were made on uninjured midribs, small pin-point size lesions appeared in some cases. On the midribs

of greenhouse plants, these lesions did not increase in size, when observed for one month. In isolations made from uninjured, inoculated midribs, after one, two, three, four and five days, in moist chambers, the fungus was re-isolated. The organism was also re-isolated from uninjured, inoculated midribs of greenhouse plants, which were plated one month after inoculation. These results indicate, that the fungus penetrated the host but did not produce the typical red rot lesions within the one month period. Observations for a longer period of time might have shown that lowered vitality of the leaves would have resulted in the production of typical red rot lesions.

Edgerton and Carvajal (35) reported that conidia of the red rot fungus, when placed on the uninjured epidermis of the leaf, germinated and produced appressoria which became cemented to the surface. They made sections through small lesions and the walls of three or four layers of cells were found reddened, but mycelium was not observed with certainty in the cells. They did not report that the infection threads penetrated the thickened walls of the epidermal layer. During the investigations reported in this paper, free hand sections were made of the uninjured, inoculated midribs and appressoria were seen on the midrib surface. In some cases, infection threads from appressoria were seen penetrating the waxy cuticle and the epidermal cells, and no reddening of the tissues was observed.

When isolations were made from uninjured, inoculated midribs four days after sterilizing in a solution of 1:1000 mercuric chloride in 50 per cent alcohol for two hours, the fungus was recovered. These results show that the fungus must have penetrated the host, where a heavy sterilization for two hours did not have any effect. Steib (53) has also

shown that the organism is not only on the surface of infected leaves, but is also in the waxy cuticle and in the tissues, where a sterilizing agent cannot reach it. Steib's results also show, that infection of bud scales from appressoria took place and a small thread was found penetrating the wall of the epidermal cells, 33 hours after inoculation.

Nesom (49) reported that infection inside the leaf sheaths takes place without injury. Edgerton and Carvajal (35) also demonstrated that infection of leaf sheaths was easily obtained by placing spore suspensions behind the leaf sheaths. During these studies, red rot lesions developed within 48 hours on inoculated uninjured leaf sheaths. The red rot symptoms appeared on the leaf blade within six or more days. On injured, inoculated leaf sheaths, symptoms developed within 24 hours, and the red rot appeared on the blade midrib within 48 hours. Nesom (49) reported that the organism would readily migrate from either part of the leaf through the ligular region. He suggested that the spread in the leaf took place in most cases by spores which were carried through the vascular bundles in the transpiration stream, since only a few pieces of the red rot bundles cultured between the lesions gave the organism. During these findings, the fungus was isolated from midrib lesions and frequently, but not always, from between the lesions. This suggests that the fungus moved from the midribs by the migration of spores and not directly by the mycelium. The source of such inoculum might be from spore suspensions, which were placed behind the leaf sheaths, or from spores produced within the cells of host tissue which migrated to the midrib through the ducts of fibrovascular bundles. Spore production within the host cells was first suggested during these studies, when

midrib lesions were first observed, appearing 20 or more days after leaf sheath inoculation. Free-hand sections were made from the diseased midribs, showing no acervuli, and atypical spores were observed in the vessels and also in parenchyma cells. A mort typical spore was also observed in the parenchyma cells on older mycelium.

Edgerton and Carvajal (34) suggested that the disease spreads in the midribs of the leaves in a manner similar to the spread in the stalks, by conidial migration in the ducts of bundles, followed by germination and production of lesions along the midribs.

Abbott (6) reported that red rot lesions a re ususally abundant in the field during the late summer and fall months. He also reported that the first leaf infections are usually noted during May or June. In the present studies, isolations of reddened midribs, collected during the early growing season, did not give the red rot fungus. However, the fungus was cultured from midrib lesions beginning in July. These results indicate that early midrib reddening is not due to the red rot fungus. Isolations from apparently healthy leaves, collected in the field, later in the season gave the red rot fungus. This suggests, that an incipient infection had taken place which did not produce typical lesions on vigorous leaves. It is suggested that a lowered vitality of older leaves, later in the season, might result in typical red rot lesions.

SUMMARY

Studies on infection and development of red rot in young shoots were made under Louisiana conditions. Leaf scars, leaf sheaths, and internal tissues of the young shoots, when cultured, gave the red rot fungus. The average percentage of red rot recovery from 1957 to 1959, from young plants of both plant and stubble cane, was 23.1 per cent from leaf scars, 28.0 per cent from leaf sheaths and 1.4 per cent from internal tissues. In some cases, up to 5 per cent of the internal tissues gave the red rot fungus.

Buds and bud scales, from portions of the shoots, which were underground, were also found infected. From all underground buds plated, the average percentage of red rot recovery was 22.3 from buds and 26.3 from bud scales.

The fungus was cultured from leaf scars and internal tissues of the underground stubble pieces of August planted cane, the shoots of which were killed during the winter.

Occurrence of red rot in young shoots, grown in the greenhouse from field inoculated and non-inoculated seed cane, was also demonstrated. Leaf scars, leaf sheaths, growing points and internal rolled leaves of the shoots of field inoculated seed cane gave red rot averages of 17.9, 5.0, 3.9, and 1.0 per cent respectively. Shoots of non-inoculated seed cane averaged 4.3 per cent from leaf scars, 5.8 per cent from leaf sheaths, 1.5 per cent from growing points and 0.2 per cent from internal rolled leaves.

From uninjured-inoculated midribs in moist chambers, the fungus recovery was 35.7 per cent after one day, 73.8 per cent after two days, 79.1 per cent after three days and 92.8 per cent after four and five days.

Effect of length of time of surface sterilization on uninjured-inoculated leaf midribs was also demonstrated. The fungus was re-isolated after sterilization in a solution of 1:1000 mercuric chloride in 50 per cent alcohol up to two hours.

Microscopic examination revealed that germinating spores, sometimes, penetrated the waxy cuticle of the upper epidermis.

When uninjured leaf midribs of greenhouse-grown plants were inoculated, symptoms of pin point size developed on some blades. In isolations, made one month later, the recovery of red rot was 60.8 per cent in Co. 290 and 68.8 per cent in C.P. 44-101. The average percentage of recovery was 58.6 per cent from upper and 71.1 per cent from lower midrib surfaces.

Where leaves were inoculated behind the leaf sheaths on greenhouse-grown plants, symptoms appeared within 48 hours and lesions on the blades were developed within six or more days. When leaf sheaths were injured by pricking with a needle, leaf sheath symptoms were observed after 24 hours and red rot lesions appeared on the blade midribs after 48 hours. The fungus was recovered from midrib lesions, from blade (lamina) lesions, and from between midrib lesions. However, isolations from between the lesions were not obtained in all cases. Hand sections of these tissues, showed the production of atypical spores within the vessels and parenchyma cells. More typical spore production was observed on older mycelium in the parenchyma cells.

When cut leaves with leaf sheaths attached and with no leaf sheaths attached were placed for four hours in a spore suspension, dispersed midrib lesions developed within 36 to 48 hours.

Development of the conidial stage of the red rot fungus inside the leaf sheaths, from field collected leaves, was demonstrated in six varieties by placing leaves in moist chambers.

The red rot fungus was isolated from apparently healthy leaves, collected in the field after June. High percentages of red rot were obtained from such leaves during late summer months.

Isolations from the reddened leaf midribs did not give the red rot fungus in all cases and especially during the early growing season. Beginning in July, however, the fungus could be cultured easily from such reddened lesions.

Isolations from between midrib lesions of field collected leaves gave the red rot fungus in 62 per cent of the isolations made.

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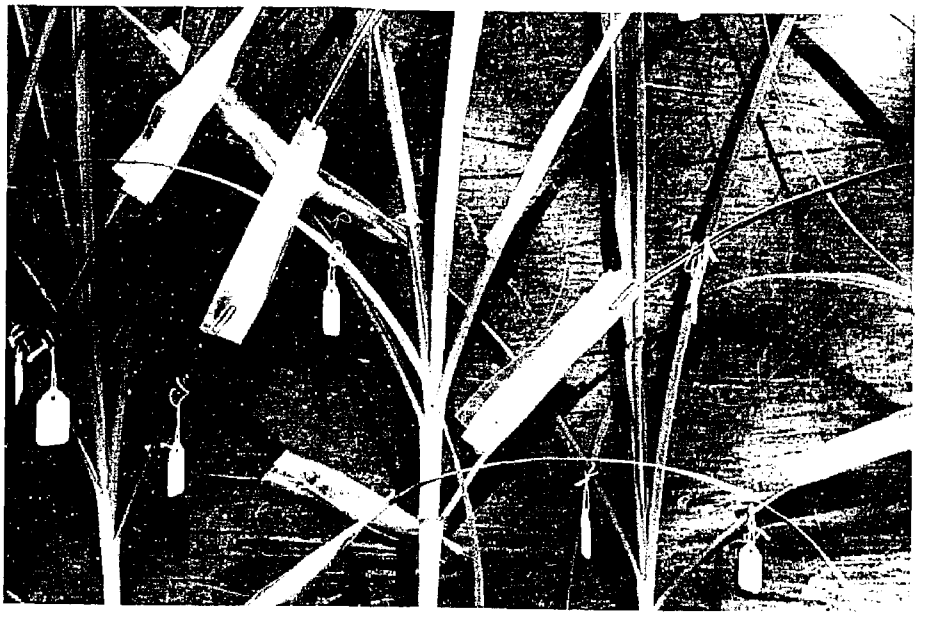


Plate I

Artificial moist chambers around leaf blades of sugarcane used for the study of midrib inoculations with red rot fungus.

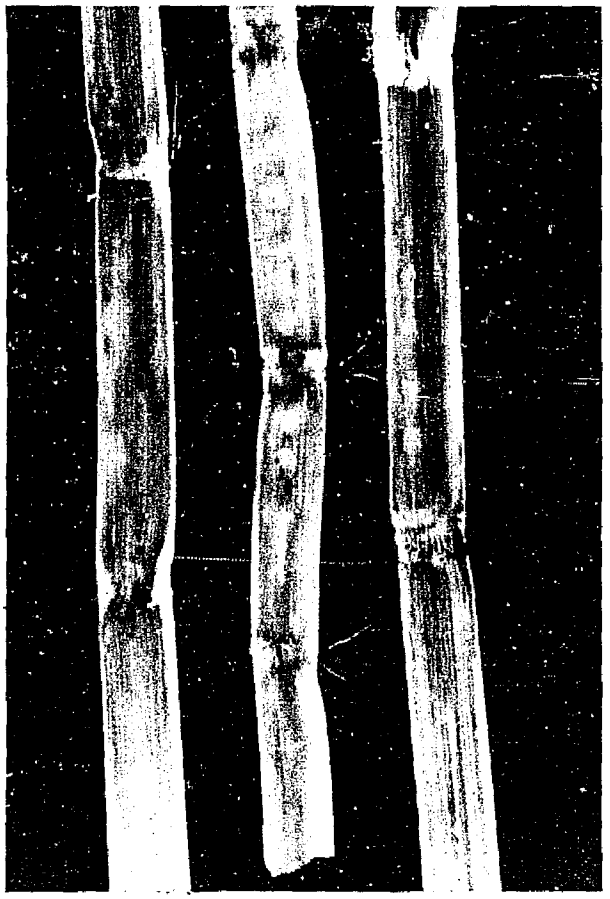
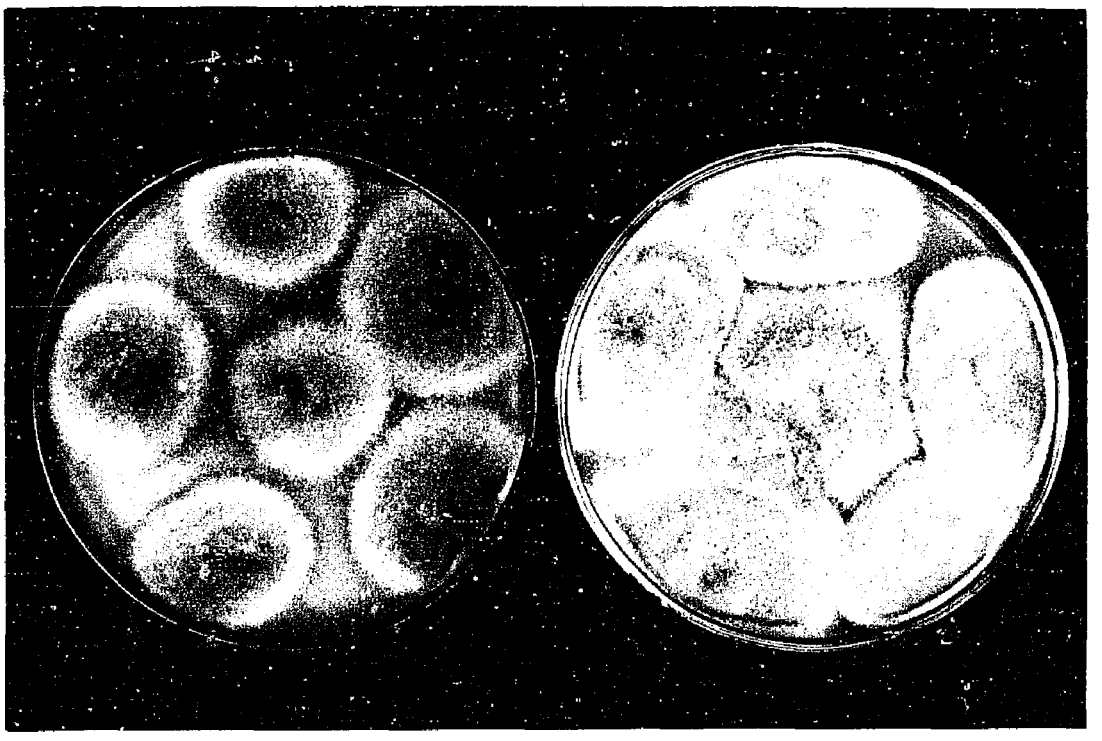


Plate II.

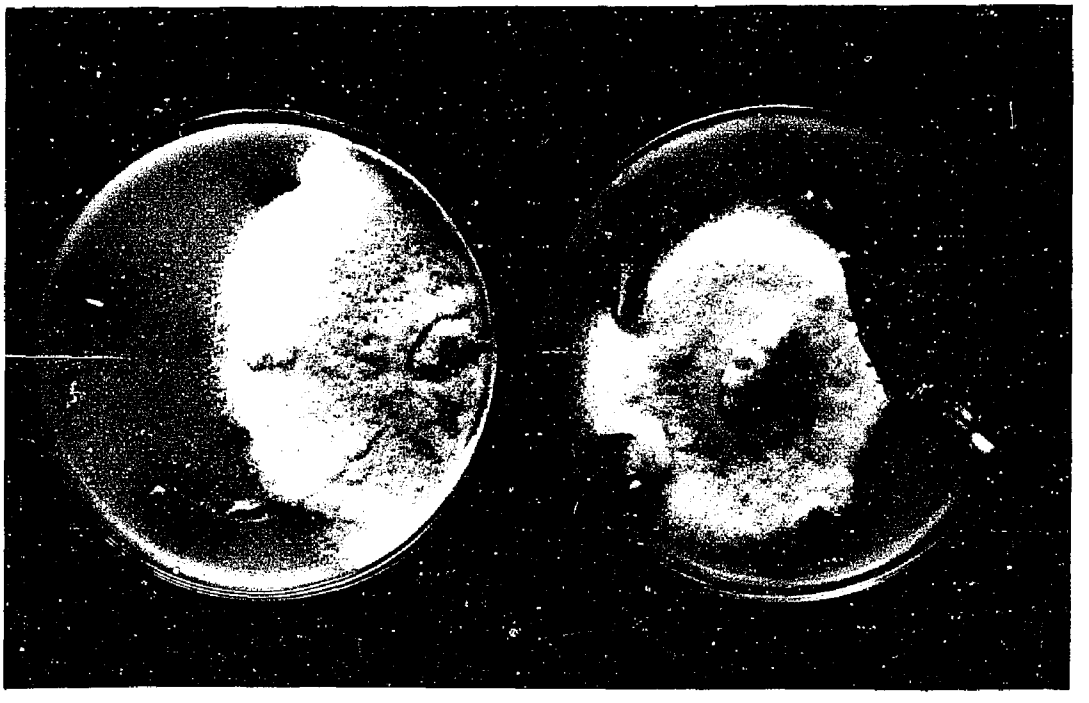
Typical internal symptoms of red rot in the variety N.Co. 310.



(1) (2)

Plate III

Pure cultures of red rot obtained from (1) leaf scars and (2) leaf sheaths of young shoots of plant cane of the variety C.P. 44-101.



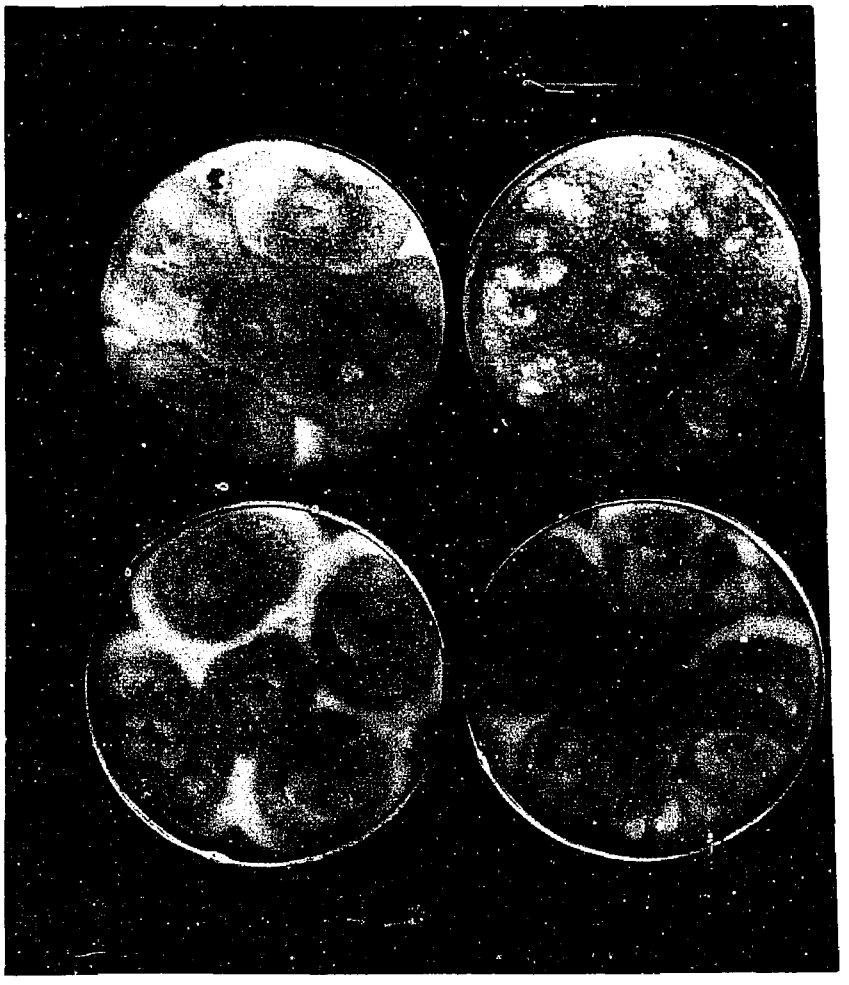
(1) (2)

Plate IV

Red rot cultures obtained from terminal growing point of young shoots. (1) C.P. 44-101, (2) Co. 290

Note: The absence of growth from sections of leaf whorl.

(1) (2)



(3) (4)

Plate V

Red rot recovered from leaf midrib inoculations in moist chambers. Top row: Co. 290; bottom row: C.P. 44-101.

(1) and (3) midribs were not injured
(2) and (4) midribs were injured

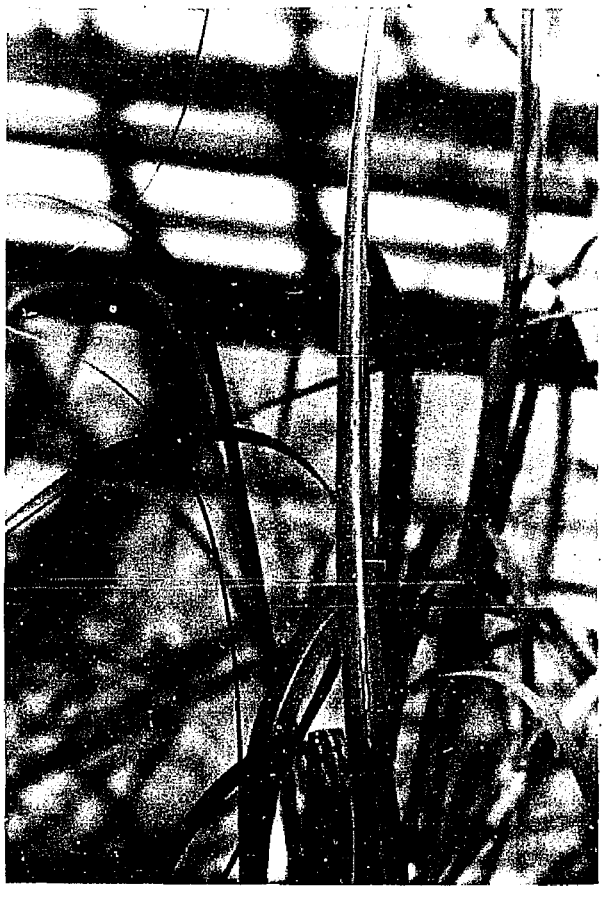


Plate VI

Greenhouse grown plants showing dispersed red rot lesions, resulting from inoculation with a spore suspension of an injured section of the basal portion of the leaf blade.

Plate VII

Greenhouse grown plants of the variety C.P. 44-101, showing red rot lesions on leaf sheaths and on the blade of leaf inouclated by placing a spore suspension back of the leaf sheath.

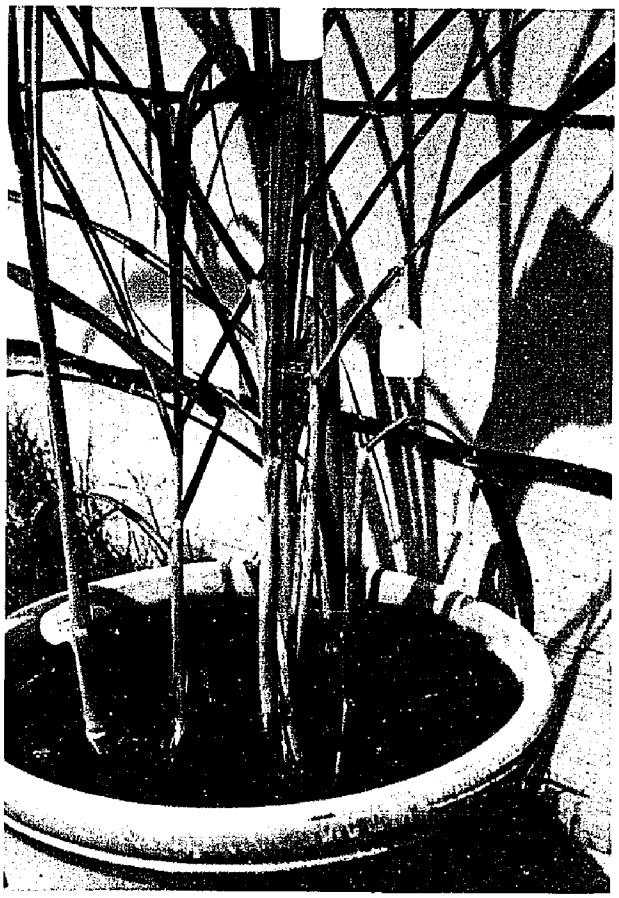


Plate VIII

Greenhouse grown plants of the variety C.P. 44-101 showing red rot lesions on the leaf sheaths and on the lower surface of the leaf midrib.

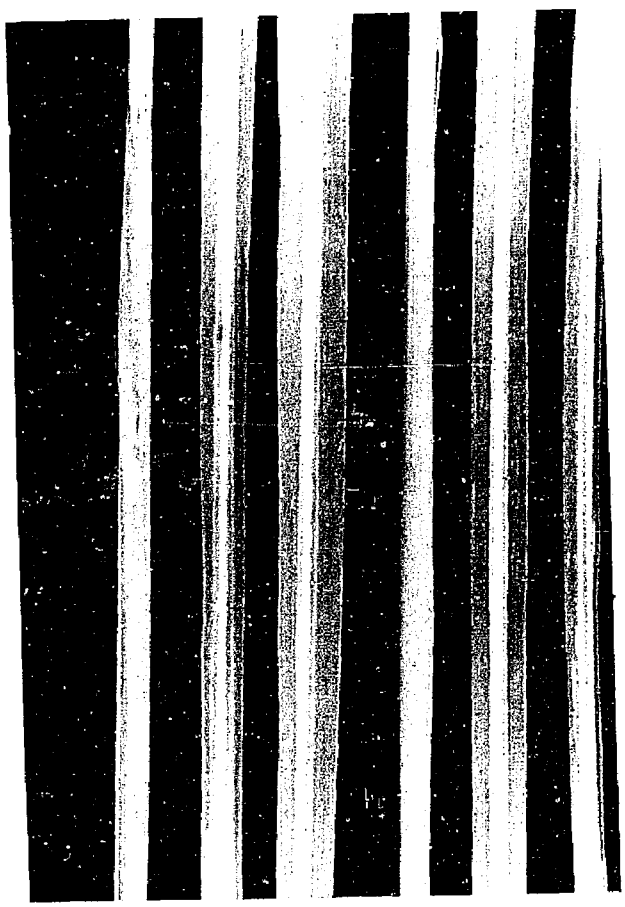


Plate IX

Early red rot symptoms on sugarcane leaf from inoculation by placing a spore suspension back of the leaf sheath.

(1) leaf sheath; (2) and (3) blade inoculated leaf.
(4) leaf sheath; (5) and (6) blade of non-inoculated check.

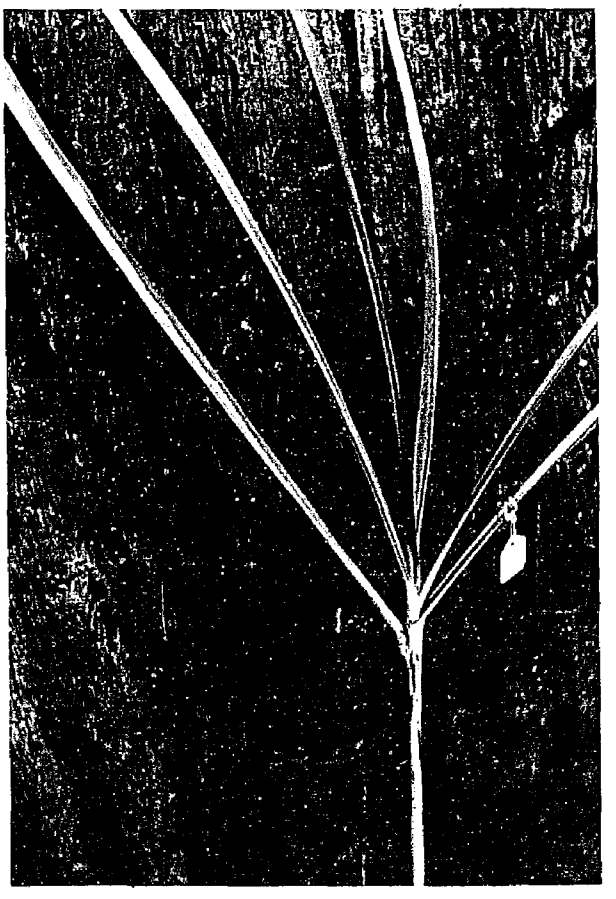


Plate X

Greenhouse grown plant of the variety C.P. 44-101 showing red rot spread into a leaf midrib from an inoculated leaf sheath.

Note: Discontinuous lesions on midrib.

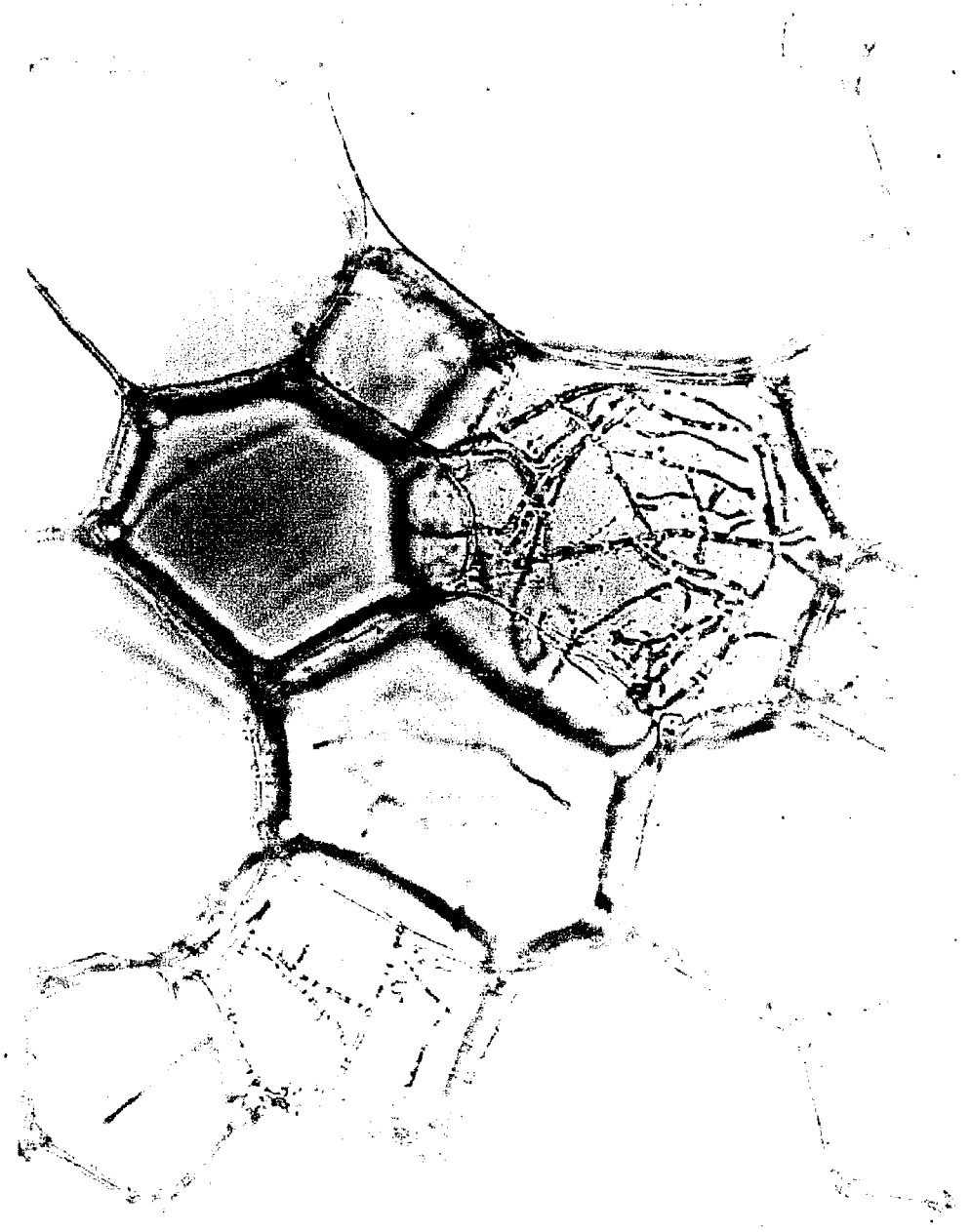


Plate XI

Section through a new red rot lesion of the midrib showing hyphae inside the parenchyma cells and spore-like structures attached to the hyphae.

Plate XII

A midrib section from a leaf sheath inoculation, showing atypical spores produced on mycelium in a parenchyma cell.

Plate XIII

Midrib section from a leaf sheath inoculation, showing spore production in a large vessel of a vascular bundle.

Plate XIV

Midrib section from a leaf sheath inoculation, showing a spore attached to a hypha in a parenchyma cell.

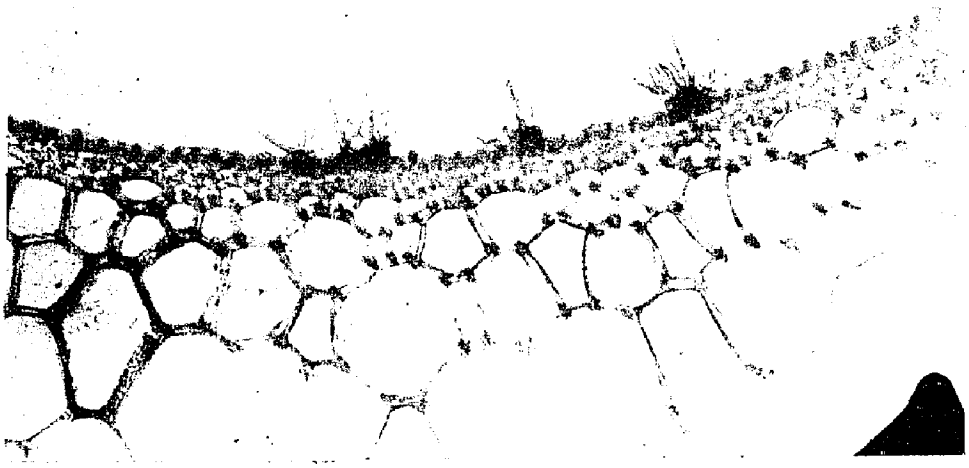


Plate XV

Section of a midrib from leaf sheath inoculation, showing acervuli with setae and spores on the upper surface of the midrib.

AUTOBIOGRAPHY

Giri Raj Singh was born on May 3, 1929 at Mahrauli (Meerut), Uttar Pradesh, India. He was graduated from the A. S. Jat High Shcool Lakhaoti (Bulandshahr) in April 1944. In July 1944, he entered the A.S. Jat College, Lakhaoti (Bulandshahr) and gradutated in April 1948 witha Bachelor of Science degree in Agriculture in First Division. In July 1948, he entered the Government Agricultural College, Kanpur and was graudated in May 1950 with a Master of Science degree in Plant Pathology in First Division, and standing First Position in order of merit.

In January 1951, he was appointed as Plant Protection Assistant in the Department of Agriculture of Uttar Pradesh, India. He worked in that position until August 1951, after then he was transferred to Govenrment Valley Fruit Research Station, Jeolikote (Nainital) as a Research Assistant in Plant Pathology. He worked at this station until December 1953. When this station moved to its previous place at Government Hill Fruit Station, Chaubattia (Almora), he moved to the new station and worked there until July 1954.

He came to the United States in September 1954 to attend the University of Wisconsin where he was granted a Graduate Research Assistantship in March 1954 in the Department of Plant Pathology. He was graduated with a Master of Science degree in Plant Pathology in August 1955. He attended the University until Janauary 1956. Because of health reasons he could not continue his studies. He entered the Louisiana State Univeristy in February 1957 and a Graduate Research Assistantship was granted to him in July of the same year. He is now a candidate for the degree of Doctor of Philosophy in August, 1959.

EXAMINATION AND THESIS REPORT

Candidate: Giri Raj Singh

Major Field: Plant Pathology

Title of Thesis: Studies on Development and Spread of Red Rot in a Sugarcane Plant.

Approved:

J. E. Forbes
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Date of Examination:

July 27, 1959